IMPERIAL AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

CONTRIBUTIONS
from
BOYCE THOMPSON INSTITUTE

VOLUME 8
1936-1937

Published Quarterly by
Boyce Thompson Institute for Plant Research, Inc.
Yonkers, New York

TABLE OF CONTENTS

VOLUME 8

No. 1, January–March, 1936

No. 2, April–June, 1936

No. 3, July–September, 1936

CONTENTS

CONTENTS

ERRATA

Page 413–430, for "microphotographs" read "photomicrographs"
Page 417, line 3, for "Two groups . . . are" read "One group . . . is"
Page 417, line 4, for "one" read "two"

ALSO NOTE ERRATA TO VOLUME 7 AS FOLLOWS

In the article "Similarities in the effects of ethylene and the plant auxins" by W. Crocker, A. E. Hitchcock, and P. W. Zimmerman, appearing in Contrib. Boyce Thompson Inst. 7(3): 231–248, 1935, there is one correction to be made which is due to the fact that Went had reported part of his results in milligrams and part of them in grams. When Dr. Crocker made the calculations he assumed that Went used the same basis throughout. As a consequence Dr. Crocker's calculations show the auxins 1000 times as effective as they are. In other words, his calculations should show ethylene 1000 times as effective as auxins *a* and *b*, figured on the molecular basis. This affects the section beginning on page 238, "Minimum effective concentrations"; also, in the "Summary" it affects paragraph 8.

Page 421, line 6, after "specimens" insert "of a related species"

Page 429, Table II, column 2, for "6.3" read "5.2"

Page 429, Table II, column 6, for "5.8" read "5.5"

In the second paragraph following Table II page 429 of this article and in the first paragraph following Table III, the percentages were calculated on the original data and the figure in Tables II and III were cut to the first decimal number.

SEEDLING PRODUCTION IN CARYA OVATA (MILL.) K. KOCH, JUGLANS CINEREA L., AND JUGLANS NIGRA L.

LELA V. BARTON

INTRODUCTION

A number of workers have reported on experiments or observations on the germination of various members of the Juglandaceae. The general results obtained have shown the need for low temperature pre-treatment. In some cases no pre-treatment has been found necessary.

Pammel and King (6) reported in 1918 that *Juglans cinerea* L. and *Juglans nigra* L. gave good germination in May or June if the seeds were planted outside the previous fall. The same treatment was effective for *Carya laciniosa* (Michx. f.) Lond. and *Carya cordiformis* (Wang.) K. Koch. On the other hand *Carya alba* (L.) K. Koch, *Carya glabra* (Mill.) Spach., and *Carya ovata* (Mill.) K. Koch could be wintered in a greenhouse and germinated in the spring.

A progress report from the Michigan Agricultural Experiment Station (5) later confirmed these results for *Hicoria ovata* and *Juglans nigra* but reported no germination either in the greenhouse or nursery for seeds of *Juglans cinerea*.

Chittenden (3) in his description of nursery and field practice recommended that *Juglans nigra* be planted in the garden in the fall.

Adams (1) obtained good germination percentages from seeds of *Juglans cinerea*, *Carya ovata*, and *Carya cordiformis* when they were sown outside in October. He used five or ten seeds for each test.

McHatton and Woodroof (4) tried different methods of storing pecans and found that those stored in a refrigerator or stratified germinated better than those from other types of storage. Plantings were made outside after storage. No description of the storage conditions was given.

According to Bailey and Woodroof (2) pecans held through the winter buried in soil germinated readily and gave the highest percentage and the most vigorous seedlings. The second best results they obtained from nuts stored in a saturated atmosphere at temperatures just above freezing.

More recently Traub and Muller (7), in their study on the effect of X-ray on pecans, secured 50 to 100 per cent germination from untreated seeds stored at 32° to 34° F. from April 16 to May 4 and then planted in sand in a cold frame.

The present paper deals with flat plantings of *Carya ovata* (Mill.) K. Koch, *Juglans cinerea* L., and *Juglans nigra* L. All of the seeds were collected before frost October 5, 1934 at Warsaw, New York. All plantings were made in flats in a mixture of peat, sand, and sod soil on October 16, 1934.

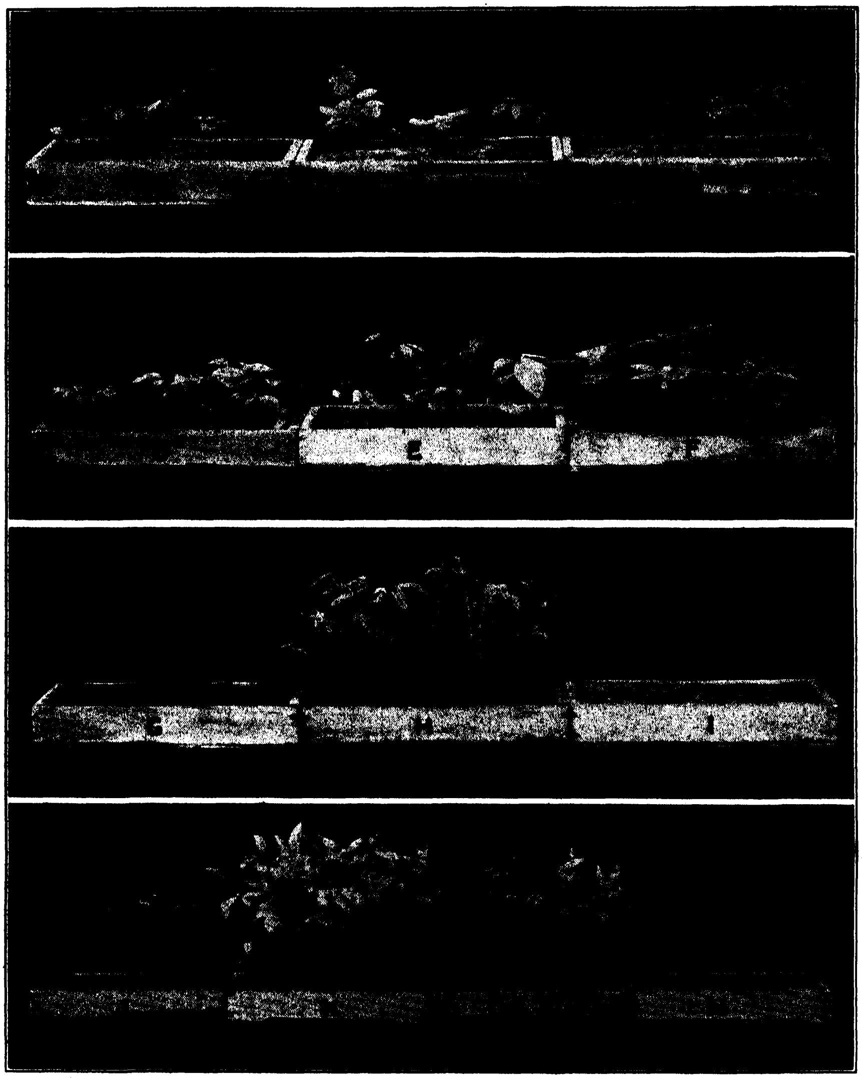


FIGURE 1. A to F. Seedling production of hickory nuts in the greenhouse. A. No low temperature pre-treatment. B, C, D, E, and F. 3° C. for 1, 2, 3, 4, and 5 months prior to transfer to the greenhouse. G to I. Seedling production of butternuts in a board-covered cold frame. G. Placed in frame in October. H and I. Transferred to frame in December and February after 2 and 4 months in a greenhouse at 21° C. J to M. Seedling production of black walnut in a board-covered cold frame. J. Placed in frame in October. K, L, and M. Transferred to frame in November, December, and February after 1, 2, and 4 months in a greenhouse at 21° C.

RESULTS AND DISCUSSION

HICKORY NUT (CARYA OVATA (MILL.) K. KOCH)

A number of flats of 50 seeds each were planted and placed in cold rooms with temperatures of 3° C. and 10° C. At monthly intervals duplicate flats were transferred from each of the cold rooms to a 21° C. greenhouse, where seedlings were produced. Seeds which were planted and placed directly in the greenhouse as controls produced only 26 per cent seedlings while a period of one month at 3° C. or 10° C. previous to transfer to the greenhouse resulted in 37 and 51 per cent seedling production. Periods up to five months at 10° C. gave practically the same results as one month. On the other hand, there was an indication of improvement in seedling production with longer periods at 3° C., especially after four and five months (Fig. 1 A to F). It will be noted in Figure 1 E and F that in spite of the fact that these flats were transferred to the germination temperature (greenhouse) one to five months later than the other flats, the prompt germination followed by rapid growth of the seedlings, made these seedlings superior in number and quality two and one-half months after the last transfer was made.

The results from outside plantings of these seeds are shown in Table I. All of these flats were planted in October 1934 and contained 50 seeds each. For the open frame, duplicate flats were used. Three flats each were used for the mulched and board-covered frames while replicates of four flats each were used for each of the greenhouse plantings some of which were transferred to the board-covered frame after two, four, and five months. Figures given in the table represent averages of the seedling production in the replicate flats for each experiment.

TABLE I

SEEDLING PRODUCTION PERCENTAGES OBTAINED FROM NUTS PLANTED IN FLATS OCTOBER 1934

Treatment in cold frames	Hickory nuts	Butternuts	Black walnuts
Open	5	—	6
Mulched	70	88	88
Board-covered	66	87	85
Board-covered after 1 mo. G.H. (21° C.)	—	—	90
Board-covered after 2 mos. G.H. (21° C.)	69	92	92
Board-covered after 4 mos. G.H. (21° C.)	42	74	90
Board-covered after 5 mos. G.H. (21° C.)	44	—	70
Greenhouse (21° C.)	26	0	0

It will be noted (Table I) that seedling production in the open frame where the flats were exposed to all of the extremes of temperature was very poor. Good seedling stands were obtained from the mulched or board-covered frames and the board-covered frames when preceded by two

months in the greenhouse. In the last case, the flats were transferred from the greenhouse to the cold frame in December. Longer periods in the greenhouse (four or five months) decreased seedling production in the board-covered cold frame. This was probably due more to the resulting short exposure to cold (transfers outside were made in February and March) than to damage by the initial high temperature period. This explanation is borne out by the results of the treatment at 3° C. (which temperature approximated that in the board-covered frame) and by the fact that even after preceding longer periods in the greenhouse, seedling production in the cold frame was superior to that in the greenhouse control (42 and 44 per cent against 26 per cent).

While pre-treatment at low temperature is not absolutely essential to the germination of hickory nut, it is decidedly beneficial in bringing about prompt, complete stands of seedlings.

BUTTERNUT (JUGLANS CINEREA L.)

Four flats each containing 25 seeds were used for each test in the experiments with butternuts. Some samples were placed in mulched and board-covered frames as soon as they were planted (October), while others were placed in the greenhouse (21° C.). Of the latter, four flats remained there throughout the experiment while others were transferred to the board-covered cold frame after two or four months. The results (Table I) show no difference in seedling production in the mulched or board-covered frame or in the board-covered frame preceded by two months in the greenhouse. Figure 1 G, H, and I, however, shows the very marked effect of the previous period at high temperature. At the time this photograph was taken (May 31, 1935) no seedlings had appeared either in the flat which had been in the board-covered frame for the entire winter or in the flat transferred from the greenhouse in February. However, a good seedling stand had already been produced in the flat with a pre-treatment of two months at a high temperature. This initial advantage later disappeared and by August seedlings from all flats were of about the same size and apparent vigor. Seeds held at the high temperature four months were apparently hampered somewhat in their germination by the subsequent short time at low temperature (only 74 per cent seedling production).

For butternuts, then, low temperature was not only effective but necessary for seedling production since without low temperature no seedlings were produced (Table I). A previous period at high temperature when followed by a sufficiently long period at low temperature hastened germination but did not increase the percentage.

BLACK WALNUT (JUGLANS NIGRA L.)

Treatment of these seeds was the same as for butternut except that pre-treatment in the greenhouse for one and five months as well as for two and four months was given. Again 25 seeds were planted in each flat.

Two flats were used in the open frame test, three flats in the mulched frame, and four flats in each of the remaining tests.

These seeds required shorter exposure to low temperature than either hickory nut or butternut since a transfer to the board-covered frame as late as February brought about good seedling production (Table I). When the transfer from the greenhouse to the board-covered frame was made in March, however, there followed a decrease in seedling production (70 per cent as against 85 to 92 per cent for other low temperature treatments). When the flats were left in the greenhouse for the duration of the experiment no seedlings were produced.

Again as for the hickory nut, the exposure in the open frame prevented seedling production (Table I). Previous periods of one or two months in the greenhouse hastened the appearance of seedlings in the board-covered frame the following spring (Fig. 1 J, K, L, M).

About two months of low temperature were essential for seedling production in this form. If the interval at low temperature was preceded by one, two, or four months at 21° C., the speed of germination was increased.

SUMMARY

Pre-treatment for two to four months at low temperatures in a moist medium was necessary for seedling production in *Juglans cinerea* and *Juglans nigra*. When a sufficiently long period at low temperature (about 3° C.) was preceded by one to four months at high temperature (21° C.), germination was hastened but not increased.

Seedling production of *Carya ovata* was improved by pre-treatment in moist soil for one to five months at 3° C. or 10° C., but some seedlings were produced without pre-treatment. A period at high temperature preceding a favorable period at low temperature had no effect.

Good seedling stands of the three forms studied were produced by fall planting when the seeds were protected by a mulch or a board cover. Exposure to freezing and thawing was harmful.

LITERATURE CITED

1. ADAMS, JOHN. The germination of the seeds of some plants with fleshy fruits. Amer. Jour. Bot. **14**: 415–428. 1927.
2. BAILEY, J. E., and J. G. WOODROOF. Propagation of pecans. Georgia Agric. Exp. Sta. Bull. 172. 22 pp. 1932.
3. CHITTENDEN, ALFRED K. Forest planting in Michigan. Michigan Agric. Exp. Sta. Spec. Bull. 163. 24 pp. 1927.
4. MCHATTON, T. H., and J. G. WOODROOF. Some factors influencing pecan germination. Proc. Amer. Soc. Hort. Sci. **24(1927)**: 125–129.
5. MICHIGAN STATE COLLEGE EXPERIMENT STATION. Progress report on study of forest tree seed, March 1926. 5 pp. 1926. (Typewritten report prepared by A. K. Chittenden and furnished through the courtesy of P. A. Herbert.)
6. PAMMEL, L. H., and C. M. KING. The germination of some trees and shrubs and their juvenile forms. Proc. Iowa Acad. Sci. **25**: 291–340. 1918.
7. TRAUB, H. P., and H. J. MULLER. X-ray dosage in relation to germination of pecan nuts. Bot. Gaz. **95**: 702–706. 1934.

GERMINATION OF SOME DESERT SEEDS

Lela V. Barton

INTRODUCTION

Spalding (3) points out that species of winter and summer annuals make up a large part of the desert flora. Plants of the winter annual type he says "begin to grow in the fall with the advent of winter rains and prevailing lower temperatures, such temperature conditions being essential to their germination, and continue until well in April, after which time they cease to be a factor in the floral covering" (3, p. 105).

MacDougal (2) speaking of the germination of winter annuals says that although the seeds remain on the ground all summer and get the summer rains they do not germinate until cooler nights come, followed by the winter rains of December. He also says that the seeds probably need "a certain length of time for the carrying out of slow changes toward maturity" (2, p. 71).

In the present study we are especially concerned with winter annuals. They complete their growth and produce seeds before the summer perennials become the characteristic vegetative feature. Such winter annuals are characteristic of southern Arizona and extend into Mexico. Some of the more important ones, among which are *Plantago fastigiata*, *Lesquerella gordoni*, and *Lepidium lasiocarpum*, often form a dense cover and are valuable for spring grazing. The period of most rapid growth is from February to April and the seeds are usually ripe in May or June (Clements 1).

Dr. Forrest Shreve of the Desert Laboratory in Tucson, Arizona, became interested in the difference in behavior of plants of the same seasonal habits. He noted the apparent tendency for a long period of drying to raise the optimum germination temperature. He also observed that, as one goes southward from Tucson, the number of winter annuals becomes smaller and some of the summer annuals of the Tucson region are found to be active in the winter season.

At the suggestion of Dr. Shreve who furnished the seed material, experiments have been conducted at this laboratory to determine the effect of air-dry storage on the germination of some winter annuals of the Tucson region. Plants studied were *Daucus pusillus* Michx., *Lepidium lasiocarpum* Nutt., *Lesquerella gordoni* (Gray) Wats., *Plantago fastigiata* Morris, and *Streptanthus arizonicus* Wats.

Preliminary experiments were conducted in 1927 and 1930 but more complete tests were made with seeds of the 1932 crop, which were received in May 1932. The results and discussion will be confined to the 1932 crop. Except in a few instances duplicates of 100 seeds each were used for each test. The figures which appear in the tables are averages of these duplicate

tests. All of the oven tests were made on moist filter paper in petri dishes while the greenhouse tests were made in a mixture of granulated peat moss, sand, and sod soil in equal parts. In case an alternating temperature was used, the cultures were left at the lower temperature for 16 hours and at the higher temperature for 8 hours each day. The greenhouse temperature was 21° C. except during the summer when the temperature could not be controlled. Germination or the appearance of the root was recorded for oven tests and seedling production or the appearance of the shoot above the soil was recorded for the greenhouse tests.

RESULTS AND DISCUSSION

Lepidium lasiocarpum Nutt. Table I shows clearly that the constant temperatures tried, as well as most of the daily alternating temperatures, were ineffective in bringing about germination of fresh seeds. A daily alternation of 10° to 30° C., however, induced germination to the extent of 21 per cent. The remaining seeds of this lot were stored air-dry at room temperature for periods of 1, 3, 6, 9, 12, 18, and 26 months. At the end of each of these periods the tests made on fresh seeds were repeated. The results (Table I) show certain definite trends in the germination of seeds stored in this manner. If the constant temperatures alone are considered, it becomes apparent immediately that 25° C. is the optimum temperature for all periods of dry storage with the possible exception of 20° C. after three months' storage. There were few seedlings produced at 10° C. or 15° C. at any time during the experiment and 35° C. proved entirely ineffective. In the three best constant temperatures, 20°, 25°, and 30° C., the highest germination percentages were obtained after the seeds had been in air-dry storage for 12 months. After this point there was a sharp decline in germination power. There was practically no germination after 18 months, but good germination was again obtained after 26 months of storage. With the exception of the greenhouse temperature which was higher when seeds were tested in July (after 26 months) than in November (after 18 months), all of the tests were made at controlled temperatures. No experimental discrepancies were observed which might account for the very poor germination after 18 months of storage.

It will be noted that for germination of fresh seeds a daily alternation of 10° to 30° C. was far superior to any other temperatures tried (21 per cent as compared with 1 to 4 per cent for other temperatures). When daily alternating temperatures were used to test stored seeds, however, the optimum storage period varied with the germination temperature used. Daily alternations of 10° to 30° C. proved more effective than any other alternation and compared favorably with a constant temperature of 25° C. In the former case, however, the highest germination was reached after one month of dry storage as compared with 12 months for the optimum

constant temperature. A daily alternation of 15° to 30° C. gave the same general results as 10° to 30° C., but the actual germination percentages were lower and the maximum was reached after three months' storage. Alternations of 10° to 20° C. and 20° to 30° C. brought about a gradual

TABLE I

Species	Temp. ° C.	% germination after storage for months							
		0	1	3	6	9	12	18	26
Lepidium lasiocarpum	Constant 10	1	4	1	3	5	3	0	4
	" 15	1	1	3	7	7	13	1	19
	" 20	0	1	10	10	27	51	1	35
	" 25	0	0	0	49	39	76	3	51
	" 30	0	0	0	2	6	25	0	12
	" 35	0	0	0	0	0	0	0	0
	Altern. 10 to 20*	4	3	10	8	10	43	1	10
	" 10 to 30*	21	74	26	17	34	42	1	22
	" 15 to 30*	3	7	33	14	12	23	0	23
	" 20 to 30*	0	0	9	22	4	49	0	49
	Greenhouse	0	1	0	3	14	2	0	17
Streptanthus arizonicus	Constant 10	2	6	9	17	19	17	6	26
	" 15	2	6	18	34	17	17	17	30
	" 20	1	2	4	18	14	20	17	25
	" 25	0	1	0	5	5	3	17	16
	" 30	1	0	0	1	1	0	1	1
	" 35	0	0	0	0	0	0	0	0
	Altern. 10 to 20*	0	3	18	29	35	36	31	51
	" 10 to 30*	0	5	20	21	26	31	54	51
	" 15 to 30*	1	1	29	38	41	28	32	42
	" 20 to 30*	0	0	0	4	1	4	36	25
	Greenhouse	0	2	3	37	47	0	33	51
Daucus pusillus	Constant 10	1	1	3	1	1	11		
	" 15	1	4	19	15	5	7		
	" 20	0	1	4	9	5	0		
	" 25	0	0	0	0	0	0		
	" 30	0	0	0	0	0	0		
	" 35	0	0	0	0	0	0		
	Altern. 10 to 20*	1	5	14	20	18	24		
	" 10 to 30*	0	10	25	28	36	43		
	" 15 to 30*	1	7	37	31	38	32		
	" 20 to 30*	0	0	0	1	0	0		
	Greenhouse	0	0	3	12	13	9		

* Kept at the lower temperatures 16 hours and at the higher temperatures 8 hours each day.

increase in germination percentage (except nine months at 20° to 30° C.) up to a maximum after 12 months of storage.

Streptanthus arizonicus Wats. After these seeds had been stored for as long as three months, alternating temperatures proved superior to constant temperatures for germination (Table I). In general, germination improved steadily, regardless of temperature, with increased length of storage up to 26 months. This was also true for seedling production in the greenhouse

with the exception of the 12-month period when, for some reason, no seedlings appeared.

Daucus pusillus Michx. Again a period of storage was advantageous for germination and daily alternating temperatures were superior to constant temperatures in bringing about germination (Table I). In this case the seed supply was exhausted after twelve months of storage.

Lesquerella gordoni (Gray) Wats. The highest germination obtained throughout the tests with these seeds was 8 per cent. It is very probable that they require special treatment of some kind for germination since they appeared sound and seemed to have good embryos. Certainly these seeds should be studied for possible coat effects or immature embryos as factors in preventing good germination.

Plantago fastigiata Morris. Seeds stored for nine months and then placed on moist filter paper at a constant temperature of 20° C. gave 27 per cent germination. This was the highest percentage obtained from these seeds. Only occasional seedlings were produced, with the exceptions of seeds stored six months and germinated at a daily alternation of 15° to 30° C., and those stored nine and twelve months and germinated at constant temperatures of 25° C. and 20° C., which conditions gave 14, 12, and 10 per cent respectively. Results here again indicated the necessity of a rest period before the seeds will germinate.

It was noticeable throughout these tests that comparatively low temperatures or combinations of low with moderately high temperatures were necessary for germination. This fact together with the dormancy exhibited by freshly-harvested seeds prevent germination during the first summer rainy season that the seeds lie on the ground. This rainy season is accompanied by day temperatures of over 100° F. in the surface layer of the soil, with but slight cooling at night (2). Such high temperatures are prohibitive to the germination of seeds of winter annuals.

There are undoubtedly some viable seeds which fail to germinate during the first winter rainy period after harvest. By the second summer rainy season, the dormancy of these old seeds has been completely overcome. Hence, the low temperature germination requirement becomes the determining factor in preventing the appearance of seedlings when moisture is supplied in July or August of the second year.

SUMMARY

A study has been made on the effect of storage on the germination temperature required by seeds of some winter annuals.

Seeds of *Lepidium lasiocarpum*, *Streptanthus arizonicus*, and *Daucus pusillus* did not germinate when they were tested immediately after harvest but as the storage time increased from 1 to 12 months, the germination percentages increased and a wider range of temperatures be-

came effective. Constant temperatures not exceeding 25° C. and as low as 10° C. were favorable. In general daily alternations of temperature, especially 10° to 30° C. and 15° to 30° C., were better than constant temperatures for inducing germination.

Lesquerella gordoni and *Plantago fastigiata* showed very poor germinations at all constant and alternating temperatures tested.

LITERATURE CITED

1. CLEMENTS, FREDERIC E. Plant indicators. The relation of plant communities to process and practice. Carnegie Inst. of Washington. Publ. 290. 388 pp. 1920.
2. MACDOUGAL, DANIEL TREMBLY. Botanical features of North American deserts. Carnegie Inst. of Washington. Publ. 99. 111 pp. 1908.
3. SPALDING, VOLNEY M. Distribution and movements of desert plants. Carnegie Inst. of Washington. Publ. 113. 144 pp. 1909.

FERTILIZER VALUE OF A NEW NITROGENOUS MATERIAL

M. M. McCool

Nitrogen in the organic form such as tankage, cotton seed meal, and others is popular as a commercial fertilizer constituent, especially in regions of high rainfall and for use on sandy soil types. Many cotton, tobacco, and vegetable growers demand that part of the nitrogen in mixed fertilizers be in this form. These carriers of nitrogen owe their popularity to the belief on the part of the users that the nitrogen in them becomes available to plants slowly and the losses by leaching of this element therefore is less than it is from the inorganic nitrogen carriers. It may be also that the presence in them of some of the rarer elements is of importance with respect to some crops when grown on soils deficient in them.

A new material,[1] which gave promise of possessing some of the above attributes, was brought to our attention by Mr. Everley M. Davis. It was considered to be advisable to determine its rate of ammonification and nitrification in soils, its effect on seed germination, early growth, and its value for crop production in comparison with high and low grade animal tankage.

In order to avoid too frequent repetitions this material will sometimes be called A; the leached product will be referred to as B.

MATERIALS AND METHODS

The ingredients employed in the manufacture of this product were untreated calcium cyanamide and concentrated residue obtained after the alcohol was distilled from fermented molasses.

The material employed in the experiments reported was obtained by mixing 100 parts of molasses concentrate and 80 parts of calcium cyanamide. It contained 14.42 per cent nitrogen, 54.9 per cent of which was water insoluble, 40.6 per cent CaO, and 2.726 per cent K_2O.

Two grades of animal tankage were employed. One carried 12.52 per cent nitrogen which was a mixture of hog and cattle scrap with the blood of the hogs in it, and the other which was taken from a bag of commercial tankage contained 7 per cent nitrogen.

The rate of formation of ammonia and nitrate from unleached and leached portions of the new product and from tankage was ascertained by mixing 0.072 gram of nitrogen in each carrier with 200 grams of Norfolk fine sand and Gloucester loam respectively. The mixtures were moistened and placed in Erlenmeyer flasks which were loosely plugged with cotton and incubated at 22° C. The ammonia and nitrate contents of two of

[1] This material was the result of researches by O. G. Stillwell of the Stillwell Laboratories, Inc., New York City, for which patent has been applied under Serial No. 718211.

each of the cultures were determined after 14, 36, and 71 days respectively. Ammonia was ascertained by dispersing 50 grams for 10 minutes in 500 cc. of 20 per cent potassium chloride. Two hundred cc. of the filtrate were placed in Kjeldahl flasks with paraffin, ground limestone, and one gram of magnesium carbonate and distilled as usual. The nitrate nitrogen present was determined by dispersing 100 grams of two of each of the cultures for 10 minutes with 500 cc. of distilled water and following the colorimetric method.

Snap bean (*Phaseolus vulgaris* L. var. Tendergreen), potato (*Solanum tuberosum* L. var. Green Mountain), cotton (*Gossypium hirsutum* L.), Turkish tobacco (*Nicotiana tabacum* L.), corn (*Zea mays* L. var. Golden Bantam), Japanese millet (*Echinochloa frumentacea* Link), and perennial rye grass (*Lolium perenne* L.) were utilized in these studies. Unless otherwise stated, glazed jars of two-gallon capacity were employed as the containers. The water losses were renewed by additions of distilled water.

The new nitrogenous product and the superphosphate employed in making the fertilizer mixtures were passed through a 20-mesh screen. The control cultures were treated with the same amount of superphosphate and potassium sulphate as was added in the mixed fertilizers. Where a portion of the nitrogen was made up of the new material or tankage the remainder consisted of equal parts of nitrate of soda and ammonium sulphate. Where a filler was required, dry sandy loam was added to the fertilizer ingredients. Unless otherwise stated in the tables of results, the fertilizers were applied in bands 1×6 inches, two inches laterally from the seed and two inches from the surface for beans; in a zone 3×3 inches, one-half inch below the potato seed pieces; in a zone 3×3 inches, two inches below the cotton seed; mixed with the surface two inches of soil for tobacco; in bands $3/4 \times 6$ inches, two inches laterally, and two inches from the surface for corn; mixed with the surface two inches of soil for rye grass; and with the entire mass of soil for millet.

EXPERIMENTAL RESULTS

Rate of formation of ammonia and nitrates. The rate of formation of of ammonia and nitrate nitrogen in Norfolk fine sand and Gloucester loam is given in Table I. There was more ammonia after 14 days in the Norfolk fine sand treated with A than there was in the other cultures to which nitrogen had been added. At the close of the experiment, or after 71 days, the ammonia nitrogen was slightly higher in the sand which had received tankage followed in order by those which contained A and B. Ammonia formed much more rapidly in the Gloucester loam soil treated with the leached and unleached new product than it did in that which had received tankage. It was highest, after 71 days incubation, in the cultures treated with B, somewhat less in those which carried A, and decidedly less in the cultures to which tankage had been added.

The Norfolk fine sand cultures which received tankage produced nitrates more rapidly than did the others and there was less of this form of nitrogen in those treated with materials A and B at the close of the experiment than there was in the controls. There were less nitrates after 36 days in the Gloucester loam soil to which the leached and unleached products were added than there were in the controls and those which had received tankage. Thirty-five days later, however, the treated cultures did not vary significantly in this respect.

TABLE I

Rate of Formation of Ammonia and Nitrate Nitrogen; P.P.M. of Soil

Treatment	Incubation period in days											
	Norfolk fine sand						Gloucester loam					
	14 days		36 days		71 days		14 days		36 days		71 days	
	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3
Control—no treatment	30	50	0	50	—	130	60	80	0	200	0	320
0.072 g. nitrogen as B	150	50	70	40	20	30	540	60	520	80	270	650
0.072 g. nitrogen as A	200	30	60	20	30	20	510	70	—	140	190	550
0.072 g. nitrogen as tankage	150	80	70	190	50	400	130	60	120	300	80	610

Experiments with snap bean. The rate and total germination of snap bean seed in Norfolk fine sand variously fertilized, the moisture contents of which were 3.1 and 8.5 per cent respectively were determined. The differences in the rate and total germination in the variously treated cultures were insignificant. It should be noted, however, that the early root development of the plants in the low moisture series of cultures was less extensive in the soil treated with fertilizer carrying the new organic material than it was in those which received a mixture containing tankage. This is illustrated by Figure 1 A.

The plants in the Norfolk fine sand of normal water content were grown until pods were well formed, harvested, and the amount of dry matter produced recorded. According to the results given in Table II the mixture in which all of the nitrogen was in the form of the new product was about one-third less efficient in the production of beans than were the other fertilizer mixtures.

Gloucester loam also was utilized as the medium in which to compare these materials. The plants were harvested when the first pods were

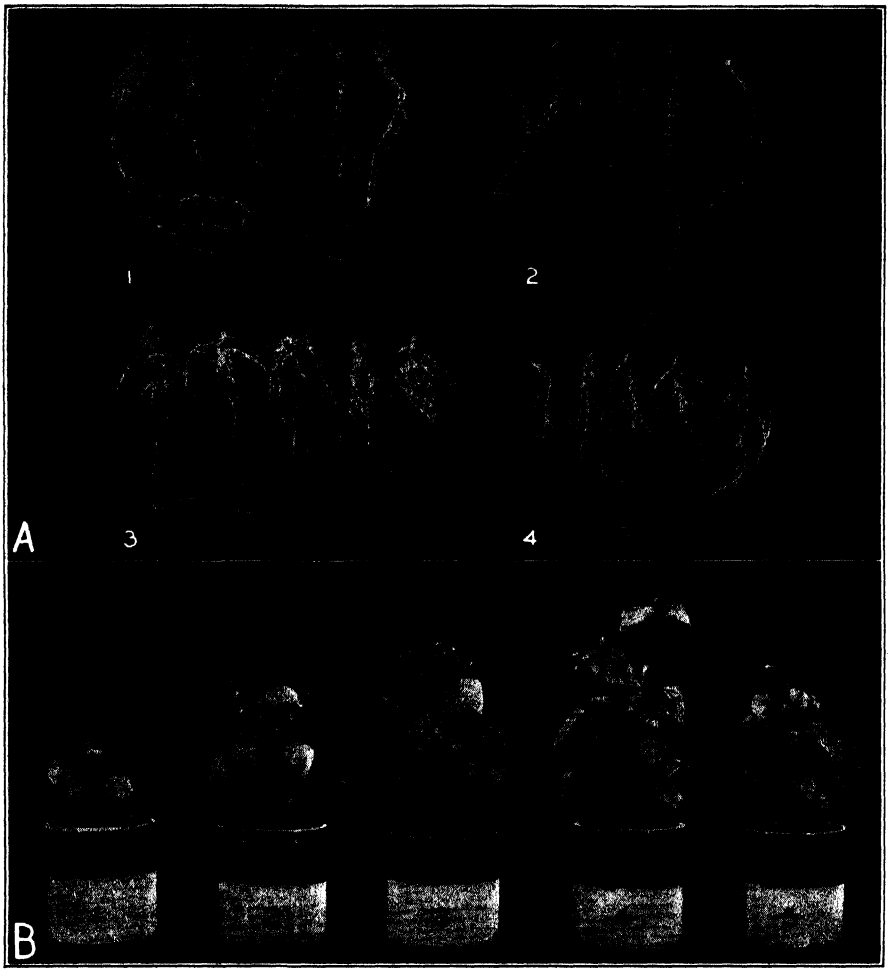


FIGURE 1. A, Roots of snap bean grown six days in Norfolk fine sand, moisture content 3.1 per cent. Rate of application of fertilizer 800 pounds per acre. Culture No. (1) P_2O_5 as in 3–8–3; (2) all nitrogen as A, 5–8–5; (3) one-half nitrogen as A, 5–8–5; (4) one-half nitrogen as tankage, 5–8–5. B, Snap bean growing in Gloucester loam. Culture No. (1) No treatment; (2) 0–8–5; (3) one-half nitrogen as A, 5–8–5; (4) one-half nitrogen as A, 5–8–5, 1600 pounds per acre; (5) one-half nitrogen as tankage, 5–8–5.

ready for picking. According to the data in Table II, illustrated in Figure 1 B, the yield in the cultures to which one-half of the nitrogen was added in product A, was larger than it was in those in which there was an equivalent amount of nitrogen in the form of tankage. It is worthy of note that the largest yields were derived from the cultures which received fertilizer in which was present the new product, at the rate of 1600 pounds per acre.

TABLE II
EFFECT OF FERTILIZERS ON THE YIELD OF SNAP BEAN GROWN IN NORFOLK FINE SAND AND GLOUCESTER LOAM

Treatment	Norfolk fine sand	Gloucester loam
	Dry wt. grams, four cultures	Dry wt. grams, five cultures
No treatment	—	16.5
P.K. as in 800 lbs. per acre, 5-8-5	16.3	31.8
800 lbs. per acre 5-8-5 1/2 nitrogen as A	—	46.0
800 lbs. per acre 5-8-5 nitrogen as A	27.09	—
800 lbs. per acre 5-8-5 1/2 nitrogen as A	37.4	—
1600 lbs. per acre 5-8-5 1/2 nitrogen as A	—	54.2
800 lbs. per acre 5-8-5 1/2 nitrogen as tankage	39.4	41.4

Experiments with tobacco. Uniform tobacco plants, two and one-half inches in length, were placed in jars of Norfolk fine sand April 8 and harvested June 8. The results obtained are summarized in Table III and illustrated by Figure 2 B. The early development of the plants to which all the cultures and one-half of the nitrogen respectively were added in A, was slightly less rapid than it was in the control cultures (Fig. 2 A). The growth of those planted in the cultures which received the heavy application of fertilizer was retarded until about three weeks after they were planted. Thereafter they grew rapidly and surpassed the remainder in yield. The differences in the yields of tobacco obtained from the cultures which received the same amount of nitrogen in the different carriers, did not vary significantly. At the time of harvest the leaves of the plants grown in cultures to which fertilizer carrying one-half the nitrogen as tankage and in those treated with superphosphate and potassium sulphate, were pale green in color, a condition due probably to nitrogen starvation.

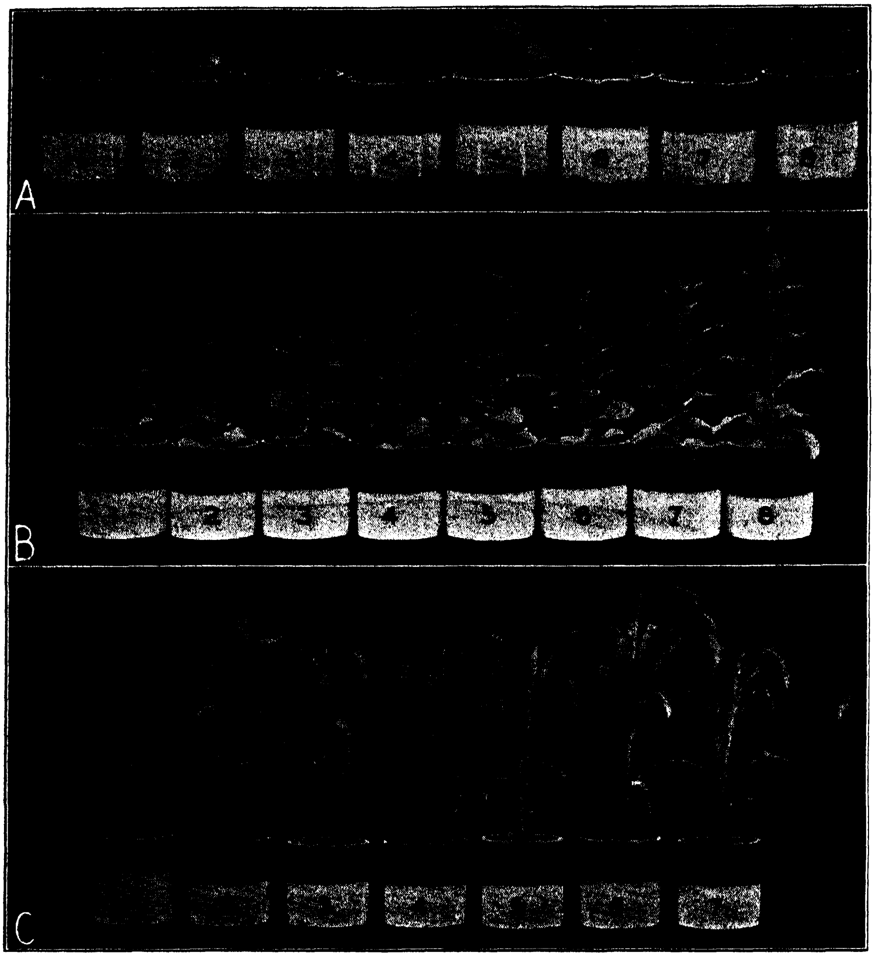


FIGURE 2. A, Turkish tobacco growing in Norfolk fine sand. Rate of fertilizer application 705 pounds per acre. Culture No. (1) No treatment; (2) P.K. as in 3–8–3; (3) all nitrogen as A (no P.K.) as in 3–8–3; (4) all nitrogen as A, 3–8–3; (5) one-half nitrogen as A, 3–8–3; (6) one-half nitrogen as B, 3–8–3; (7) one-half nitrogen as tankage, 3–8–3; (8) one-half nitrogen as A, 3–8–3, 1410 pounds per acre. B, Tobacco growing in Norfolk fine sand. Culture No. (1) No treatment; (2) P_2O_5 K_2O as in 3–8–3; (3) all nitrogen as A (no P.K.) as in 3–8–3; (4) all nitrogen as A, 3–8–3; (5) one-half nitrogen as A, 3–8–3; (6) one-half nitrogen as B, 3–8–3; (7) one-half nitrogen as tankage, 3–8–3; (8) one-half nitrogen as A, 3–8–3, 1410 pounds per acre. C, Corn growing in Gloucester loam. Rate of fertilizer application 300 pounds per acre. Culture No. (1) P.K. as in 3–8–3; (2) all nitrogen as A in 3–8–3; (3) one-half nitrogen as A in 3–8–3; (4) one-half nitrogen as insoluble A in 3–8–3; (5) one-half nitrogen as tankage in 3–8–3; (6) same as culture 3, 600 pounds per acre; (7) same as culture 4, 600 pounds per acre.

Gloucester loam also was employed as the medium in which to compare these materials. Unlike the previous results obtained from the Norfolk fine sand cultures there was no retardation of the early growth of these plants. The total dry weights obtained from the cultures which received the same amount of nitrogen in the different carriers did not vary beyond the limits of the experimental error. It was observed, however, that the leaves of the plants grown in the soil to which the high grade tankage was applied were smaller and lighter green in color than were those produced in the cultures which received the new product. The results obtained are presented in Table III.

TABLE III
Effect of Fertilizers on the Growth of Tobacco

Culture number	Treatment	Norfolk fine sand	Gloucester loam
		Dry wt. in grams, three cultures	Dry wt. in grams, five cultures
1	No treatment	9.0	—
2	P.K. as in 705 lbs. per acre 3–8–3	12.6	18.3
3	Nitrogen as A, 705 lbs. per acre 3–0–0	16.3	—
4	Nitrogen as A, 705 lbs. per acre 3–8–3	19.3	30.1
5	1/2 nitrogen as A, 705 lbs. per acre 3–8–3	22.1	29.2
6	1/2 nitrogen as tankage, 705 lbs. per acre 3–8–3	22.2	32.1
7	1/2 nitrogen as A, 1410 lbs. per acre 3–8–3	29.9	60.8
8	1/2 nitrogen as B, 705 lbs. per acre 3–8–3	—	30.7

Experiments with corn. Gloucester loam was employed as the medium in which to grow corn. The seeds were planted April 17 and the resulting plants harvested June 7. There was no difference in either the rate or total germination of the seed in the differently treated soil cultures. As the data in Table IV show, the different carriers of nitrogen were equally effective. The application of 3–8–3 fertilizer, in which one-half of the nitrogen was in the form of A at the rate of 600 pounds per acre, resulted in the largest yield of corn (Fig. 2 C).

Studies on the germination and early growth of potato. The potato seed pieces were carefully selected and so prepared that they contained approximately the same amount of tissue and were placed one-half inch

above the fertilizer, with the sprout-producing portion perpendicular to it. The rate and total number of sprouts that appeared in Norfolk fine sand of 2.8 and 8.5 per cent water were observed. The containers which carried the sand with the low water content were kept covered to prevent loss of water, since it was impossible to add water without raising the water content above the original. Two seed pieces were placed in each jar of soil. Twelve cultures of each fertilizer treatment were made up. There was a slight delay in the appearance of the sprouts at the surface of the sand which had been fertilized with the mixture in which one-half of the nitrogen was present in the form of the new material.

The dry weight of the plants produced in the sand cultures which contained 8.5 per cent water was determined after 30 days. The yields obtained were as follows: control 41.5 grams, one-half the nitrogen as new

TABLE IV

EFFECT OF FERTILIZERS ON THE GROWTH OF CORN IN GLOUCESTER LOAM

Treatment	Yield dry wt. in grams
P.K. as in 300 lbs. per acre 3–8–3	14.5
Nitrogen as A, 300 lbs. per acre 3–8–3	22.5
1/2 nitrogen as A, 300 lbs. per acre 3–8–3	23.5
1/2 nitrogen as A, 600 lbs. per acre 3–8–3	55.6
1/2 nitrogen as tankage, 300 lbs. per acre 3–8–3	23.7

material 70.2 grams, and one-half the nitrogen as tankage 63.4 grams. The results are interesting in that the effect of the factor or factors concerned in the delay of the appearance of the sprouts was obliterated later on.

Experiments with cotton. Ten carefully selected cotton seed were placed in each culture of Norfolk fine sand. Each soil treatment was replicated six times. The differences in the rate and total germination of the seed in the variously treated cultures were insignificant. At the close of the germination period the number of plants was reduced to three in each culture. Twenty days after planting, the root development in the various cultures was observed. They were normal in color but those in the control cultures were about twice as extensive as were those in the others.

Sixty days after sowing the seed, two plants were removed from each culture and the dry weights determined. According to the data in Table V, the fertilizer mixture which contained one-half of the nitrogen as product A was superior to the one which carried one-half of the nitrogen as tankage, irrespective of the amounts applied or the method of application. The cultures to which all of the nitrogen was applied in the form of the new organic nitrogenous material had produced less dry matter than had those which received the other mixtures. It was evident that the nitrogen in this carrier was more slowly available than it was in the tank-

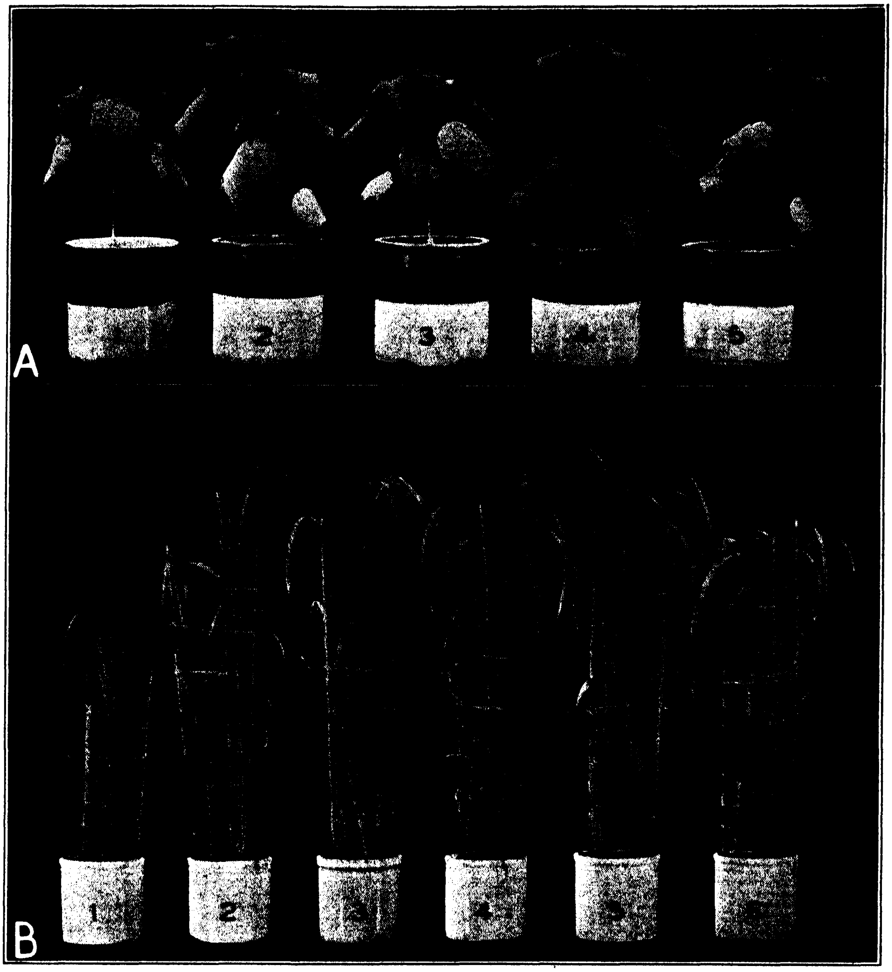


Figure 3. A, Cotton growing in Norfolk fine sand. Rate of fertilizer application 600 pounds per acre. Culture No. (1) 0–8–4; (2) all nitrogen as A in 4–8–4; (3) one-half nitrogen as A in 4–8–4; (4) one-half nitrogen as B in 4–8–4; (5) one-half nitrogen as tankage in 4–8–4. B, Japanese millet growing in Norfolk fine sand. P_2O_5 K_2O as in 10 grams of superphosphate and 1/2 gram KCl. Culture No. (1) P_2O_5 K_2O; (2) P_2O_5 K_2O+0.072 gram nitrogen as A; (3) P_2O_5 K_2O+0.144 gram nitrogen as A; (4) P_2O_5 K_2O+0.288 gram nitrogen as A; (5) P_2O_5 K_2O+0.241 gram nitrogen as B; (6) P_2O_5 K_2O+0.144 gram nitrogen as tankage.

age. Subsequently, however, the cotton plants in the cultures which carried the former outgrew those in the soil to which tankage was added as shown by the data in Table V and illustrated by Figure 3 A. At the close of the experiment the plants growing in the cultures which contained carriers A and B were darker green in color than were those in the other cultures.

TABLE V
EFFECT OF DIFFERENT NITROGEN CARRIERS ON THE GROWTH OF COTTON; NORFOLK FINE SAND

Culture number	Treatment	Yield dry wt. in grams, 12 plants after 60 days	Yield dry wt. in grams, 6 plants after 110 days
1	0-8-4*	24.8	18.2
2	All nitrogen as A	33.2	36.1
3	1/2 nitrogen as A	42.8	32.6
4	1/2 nitrogen as leached A	—	43.2
5	1/2 nitrogen as A	54.2	33.2
6	1/2 nitrogen as A	56.8	48.2
7	1/2 nitrogen as tankage	49.4	42.4
8	1/2 nitrogen as tankage	36.4	27.3

* Fertilizer applied in 3×3 inch zone below seed at the rate of 600 pounds per acre of 4-8-4 in cultures 1, 2, 3, 4, and 8 and in bands 1×6 inches in cultures 5, 6, and 7. Fertilizer added at the rate of 1200 pounds per acre in cultures 6 and 7.

Experiments with millet. Japanese millet was grown in Norfolk fine sand and Gloucester loam. In this series, the fertilizer value of the nitrogen in the unleached and leached new nitrogen carrier and in tankage was studied. The yields were taken as the seed were forming. The results obtained are given in Table VI and illustrated by Figure 3 B. The loam soil cultures to which the new material was added produced 10.9 per cent more dry plant material than did those which received the same amount of nitrogen in the form of tankage. The yield derived from the sand cultures which carried the product was 23.6 per cent greater than was that pro-

TABLE VI
FERTILIZING VALUE OF NITROGEN IN A, B, AND TANKAGE; GROWTH INDICATOR, JAPANESE MILLET

Culture number	Treatment	Dry wt. in grams, 21 plants	
		Gloucester loam	Norfolk fine sand
1	P.K.	36.0	9.9
2	P.K.+0.072 gram nitrogen as A	50.5	23.9
3	P.K.+0.144 gram nitrogen as A	56.9	37.6
4	P.K.+0.288 gram nitrogen as A	54.0	41.8
5	P.K.+0.241 gram nitrogen as B	64.7	37.8
6	P.K.+0.144 gram nitrogen as tankage	51.3	30.4

TABLE VII
Rate of Availability of Nitrogen in B and in Tankage; Growth Indicator, Rye Grass

Treatment	Fresh weight in grams from three cultures					
	June 6	June 17	July 1	July 18	Aug. 3	Total
P.K.*	35.0	33.1	23.8	13.5	8.0	113.4
P.K.+0.132 gram nitrogen in B	35.5	51.2	51.1	21.2	14.5	173.5
P.K.+0.396 gram nitrogen in B	43.5	50.0	53.5	33.2	20.0	200.2
P.K.+0.268 gram nitrogen in tankage	47.5	50.5	42.0	18.6	10.2	168.8

* 10 grams superphosphate and 1/2 gram KCl.

duced by the cultures which were treated with the same amount of nitrogen in the form of tankage.

Rate of availability. Rye grass was grown in one-gallon jars filled with Gloucester loam, in order to compare the fertilizing value and the rate of availability of the water insoluble nitrogen in the new material and in tankage. The yields of each of five cuttings from the differently fertilized cultures were ascertained. According to the data in Table VII, 0.132 gram of nitrogen in the leached new product produced, within the limits of experimental error, the same yields as did 0.268 gram in tankage. The early growth effects of the tankage were greater than were those of the leached nitrogen carrier, but the yields obtained from the third and fourth

TABLE VIII
Availability of Nitrogen Carriers; Dry Weight in Grams of Three Cultures of Rye

Treatment	Period of growth in days					
	Gloucester loam			Norfolk fine sand		
	35	63	91	35	63	91
P.K.*	1.57	6.4	7.50	3.10	9.80	10.85
P.K.+0.14 g. nitrogen as A	4.62	14.6	18.41	5.70	14.93	19.51
P.K.+0.14 g. nitrogen as B	4.55	11.3	15.32	4.15	16.63	19.09
P.K.+0.14 g. nitrogen as high nitrogen tankage	3.51	10.4	12.30	4.95	15.42	19.27
P.K.+0.14 g. nitrogen as low grade tankage	4.31	11.6	13.0	3.80	15.45	19.32

* 10 grams superphosphate and 1/2 gram KCl.

cuttings were less from the tankage-treated cultures than they were from those to which product B was applied.

Rye was also employed as an indicator for comparing the rate of availability of unleached and leached new fertilizer and tankage. Gloucester loam and Norfolk fine sand cultures were employed in these tests. Each cultural treatment was replicated three times for each growth period. According to the data in Table VIII the yields at the close of each growth period, produced by the Gloucester loam cultures fertilized with the tankage carrying a high per cent of nitrogen, were less than those derived from the other cultures which had received nitrogen. The new organic nitrogenous material was superior to the other nitrogen carriers after nine weeks and after thirteen weeks the cultures treated with it and the leached product had produced more plant material than those which had received tankage.

The variations in the yields derived from the nitrogen-treated Norfolk fine sand cultures, with the exception of those produced in the cultures fertilized with low grade tankage at the termination of the first growth period, were insignificant.

SUMMARY OF RESULTS

Ammonia was formed more rapidly from the unleached new material than it was from tankage in the Norfolk fine sand. At the close of the incubation period, however, the ammonia content was greatest in the Norfolk fine sand to which tankage had been added. Ammonification of tankage took place less rapidly in Gloucester loam than did that of products A and B and there was also less ammonia present after 71 days in the tankage-treated soil.

Nitrification of tankage was more rapid in Norfolk fine sand than the other carriers of nitrogen. The new material whether leached or not depressed somewhat the rate of formation of nitrates. The same was true in the Gloucester loam after 36 days but after 71 days the nitrogen-treated soils did not vary significantly in this respect.

It did not decrease the percentage and rate of germination of snap bean, corn, and cotton, but delayed slightly the rate of the appearance of sprouts from potato cuttings. It was the equal of high grade tankage for the production of snap bean, tobacco, corn, and was superior to it for the production of cotton, rye grass, and millet. Owing to the slower rate of availability, the losses entailed through leaching under field conditions should be less than from tankage or inorganic carriers of nitrogen.

CARBON DIOXIDE STORAGE. IX. GERMINATION OF LETTUCE SEEDS AT HIGH TEMPERATURES IN BOTH LIGHT AND DARKNESS

NORWOOD C. THORNTON

The germination of recently harvested lettuce (*Lactuca sativa* L.) seeds is controlled by many conditions such as temperature, moisture supply, aeration, and light. Lettuce seeds germinating perfectly at temperatures below 20° C. will not germinate at a temperature of 35° C. even when all other conditions for germination are considered to be favorable. Davis (2) pointed out that the reason for the poor germination of lettuce seeds at many temperatures was due to lack of sufficient moisture and aeration. He suggested the use of moist cotton as the substratum for germination since it supplied abundance of moisture without hinderance to proper aeration of the seeds. Davis also recognized that these suggestions did not provide a solution of the problem of high temperature germination. During his investigations he found that the rate of respiration of the lettuce seeds was markedly reduced with an increase in temperature. From these results he concluded that the moist seed coats become less permeable to oxygen with the increase in temperature of the germinator; thus a dormant condition was developed. Davis pointed out that seeds remaining in the germinator at a high temperature for many months without germinating could be made to germinate only by removal of the seed coats or by lowering the temperature of the germinator.

Borthwick and Robbins (1) were able to germinate lettuce seeds at high temperatures by first subjecting the moist seeds to low temperature in order to initiate germination. They also considered that the seed coats inhibited the passage of oxygen to the embryo at high temperatures. As Rose (6) had previously done they subjected lettuce seeds to increased oxygen pressures at 30° C. with the result that considerable germination of the seeds was obtained.

Shuck (7) in his studies of the factors influencing the germination of lettuce seeds concluded that a moist substratum of cotton, low temperature, and exposure to light are needed for germination. Both he and Flint (3) have shown conclusively the beneficial action of light upon the germination of lettuce seeds at high temperatures. Shuck (8) considers that light may aid in the alteration or removal of some substance which acts as an inhibitor of germination of the seeds held at the high temperatures.

In a recent report (9) from this laboratory it was shown that germination of the seeds of the cocklebur could be forced or hastened by the presence of carbon dioxide in the germinator. Especially noticeable was the germination of the upper seeds in mixtures of carbon dioxide and oxygen

at 25° C., a temperature considerably below that necessary for the germination of the seeds under normal atmospheric conditions. In this paper further evidence of the physiological action of carbon dioxide on plant tissue is reported.

Germination of recently harvested lettuce seeds may be obtained readily in either light or darkness at temperatures as high as 35° C. if the moist seeds are exposed to mixtures of carbon dioxide and oxygen.

TABLE I

VARIETY NAME AND HARVEST DATE OF THE LETTUCE SEEDS USED IN THIS INVESTIGATION

Variety	Variety number	Harvest date
	White-seeded	
Early Curled Simpson	5130	Aug. 27, 1935
White Boston	5138	Aug. 23, 1935
Big Boston	5146	Sept. 2, 1935
New York No. 12	3099	1933
	Black-seeded	
Mammoth Black-Seeded Butter	5140	Aug. 24, 1935
Black-Seeded Simpson	5164	Sept. 4, 1935
Black-Seeded Big Simpson	5284	Oct. 10, 1935

MATERIAL AND METHODS

This investigation was conducted during December, January, and February upon seeds of both white- and black-seeded varieties of lettuce that were obtained early in December from the Ferry-Morse Seed Co.[1] The names of the varieties of lettuce seeds used in these tests together with the variety number and harvest date of each variety are given in Table I.

Absorbent cotton saturated with distilled water was used as a substratum for all the germination tests in either petri dishes or 500 cc. Erlenmeyer flasks. The lettuce seeds were exposed to the various gas mixtures in the dark by placing the petri dishes, without covers, in 8 liter tin cans and in the light by holding the seeds in Erlenmeyer flasks. The gas mixtures were made up over water with the gases which were obtained from commercial cylinders of oxygen, carbon dioxide, and nitrogen. The gas mixtures were flowed into and through the cans and flasks by means of inlet and outlet tubes sealed in the top of the former and in the cork of the latter. The tin can top was sealed on with paraffin and the cork was sealed in the neck of the flask with Dekhotinsky cement so that all open-

[1] The writer wishes to express his appreciation to Mr. Frank G. Cuthbertson, Vice-President of Ferry-Morse Seed Co., San Francisco, Calif., for his interest in making available the seeds and information on harvest dates.

ings in the germinators were closed after the gas mixtures were applied at atmospheric pressure.

The seeds during the germination tests were held in constant temperature ovens and rooms with the exception of room temperature which varied from 24° to 27° C. with a daytime average of 26° C. and occasional night temperature of 24° C.

EXPERIMENTAL RESULTS

GERMINATION IN ABSENCE OF CARBON DIOXIDE

The data in Table II show that the germination of recently harvested lettuce seeds is inhibited by either or both high temperature or darkness and is favored by low temperature and light. Seeds exposed to high temperatures, 30° to 35° C., are thrown into a condition of dormancy from which the seeds will not emerge until either the seed coat is removed

TABLE II

EFFECT OF TEMPERATURE AND LIGHT ON THE GERMINATION OF LETTUCE SEEDS

Lettuce seed var. No.	Percentage germination at various temperatures during definite periods							
	72 hrs. at 5° C., then	20° C.	26° C.		30° C.		35° C.	
	48 hrs. at 20° C.	48 hrs.	48 hrs.	96 hrs.	48 hrs.	96 hrs.	48 hrs.	96 hrs.
	In light							
5130	98	95	25	66	1	1	0	0
5138	100	99	54	83	4	6	2	4
5146	100	100	51	89	8	8	0	0
5140	100	100	97	100	38	39	16	21
5164	100	98	97	98	45	45	0	0
5284	98	96	95	95	87	89	23	24
	In darkness							
5130	97	95	2	24	0	0	0	0
5138	99	93	0	10	0	1	0	1
5146	100	89	0	1	0	0	0	0
5140	98	100	9	35	11	13	16	22
5164	99	98	1	14	0	0	0	0
5284	99	96	6	17	0	0	0	1

or the temperature is lowered. This condition is not due however to seeds of poor vitality since it may be seen in Table II that samples of the same lots of seeds gave practically complete germination when held at a temperature of 20° C. or lower. Furthermore the dormant condition is not a problem of slower germination of the seeds held at the high temperature since there is very little change in the percentage of germination after the first 48 hours in the germinator. Even those seeds giving a fair percentage of germination at 30° C. are markedly if not completely inhibited

in germination when the temperature is raised to 35° C. even in the presence of light.

Some varieties of seeds are affected more than others by the high temperatures and the response in germination can not always be correlated with age of the seed. An example of such a fact is readily seen in the similar percentage germination of the two black-seeded varieties 5140 and 5284 held in light at 35° C. The seeds of these two varieties, 5140 and 5284, represent the earliest and latest harvested seeds respectively of those used in these tests.

Light favors to some extent the germination of many of the varieties of lettuce seeds held at the high temperatures. As shown in Table II five of the six varieties of lettuce seeds were inhibited from germinating when held in complete darkness at 30° or 35° C. Even at 26° C. very little germination of the seeds was obtained in darkness during 48 hours and from 1 to 35 per cent germination was obtained within 96 hours. During these same periods the lettuce seeds in light at 26° C. gave very high percentage germination. However, at the lower temperature, 20° C., light was not found to be necessary for the germination of the lettuce seeds. Just what effect light has upon these seeds is not yet known.

The dormancy exhibited by the lettuce seeds held in the germinators at high temperatures is not due primarily to a condition of the embryo. Imbibed lettuce seeds held at 26°, 30°, or 35° C. will germinate within 24 hours if care has been exercised to remove completely the seed coats without injury to the embryo. Furthermore, intact seeds that have been held in the germinator at 35° C. for six days will germinate at this temperature within 48 hours after the seed coats are removed. Also these seeds, dormant in light at 35° C., will germinate in the dark at 35° C. provided that the seed coats are broken or removed while in complete darkness. These results indicate that the seed coat is the controlling factor in the development of dormancy of the lettuce seeds held in germinators at high temperatures.

GERMINATION IN LIGHT IN THE PRESENCE OF CARBON DIOXIDE

White-seeded varieties. Lettuce seeds that are unable to germinate at temperatures above 20° C. may be forced to germinate by exposure to carbon dioxide gas in the presence of oxygen. For example, the data in Table III show that the white-seeded varieties of lettuce seeds exposed to as little as 5 per cent or even 10 per cent of carbon dioxide for 17 hours at 26° C. germinated far better than the control seeds. The Early Curled Simpson variety No. 5130, which is the hardest to germinate of the seeds used in these experiments, gave 31 per cent germination in 5 per cent carbon dioxide as compared with zero germination for the control. With the increase in the germination period to 65 hours one finds that 80 per

TABLE III

GERMINATION OF WHITE-SEEDED VARIETIES OF LETTUCE SEEDS IN CARBON DIOXIDE WITH 20 PER CENT OF OXYGEN*

Variety No.	Temp. °C.	Hrs. of treatment	Percentage germination in the following percentages of carbon dioxide						
			0	5	10	20	40	60	80
5130	26	17	0	31	23	5	0	0	0
		41	0	40	36	7	0	0	0
		65	0	43	40	11	5	53	66
	30	17	0	0	0	0	0	0	0
		41	0	0	0	0	0	13	27
		65	0	0	0	0	6	28	85
		113	2	0	0	0	6	73	95
	35	17	0	0	0	0	0	0	0
		65	1	0	1	0	1	17	8
		89	1	0	1	0	3	26	25
		137	1	0	1	0	5	78	80
5138	26	17	7	11	24	4	0	0	0
		41	10	26	31	24	27	38	24
		65	12	29	36	28	55	70	87
	30	17	5	4	2	3	0	0	0
		41	9	5	4	8	25	37	60
		65	9	6	5	8	44	73	90
		113	10	6	5	11	49	85	95
	35	17	0	0	1	0	0	0	0
		65	1	1	5	1	11	33	14
		89	1	1	5	1	12	61	56
		137	1	5	9	4	28	90	99
5146	26	17	7	20	21	9	0	0	0
		41	10	49	42	57	23	10	7
		65	10	55	45	68	47	91	98
	30	17	0	0	0	0	0	0	0
		41	0	0	0	6	18	17	51
		65	0	0	0	6	27	70	80
		113	3	1	1	6	32	88	98
	35	17	0	0	0	0	0	0	0
		65	0	0	0	1	2	18	4
		89	0	0	0	1	7	61	35
		137	1	0	2	1	7	91	83

* The seeds were exposed to considerable daylight during these tests.

cent of carbon dioxide forced 66 per cent germination as compared to zero for the control seeds of this variety 5130. Seeds of the other two varieties gave almost complete germination in carbon dioxide when held for 65 hours at 26° C.

With an increase in the temperature of the germinator to 30° C. one finds that two factors must be increased. These factors are the percentage

of carbon dioxide necessary to force the seeds to germinate and the period that the seeds must be exposed to the gas before germination begins. As shown in Table III, the seeds exposed to 30° C. require from 40 to 80 per cent of carbon dioxide and a period of treatment of approximately 41 hours to force about the same percentage of germination as 5 to 10 per cent of carbon dioxide did within 17 hours at 26° C. As the period of carbon dioxide treatment of the seeds is extended there is a continual increase in the percentage germination until practically complete germination is obtained. These results are to be compared with 2 to 10 per cent germination of the control lots of seeds during the same period as the treated seeds in the germinators at 30° C.

A further increase in the temperature of the germinators to 35° C. brought about the necessity for a still longer period of treatment of the seeds with carbon dioxide. The results show that about 65 hours are required for the high (40 to 80) percentages of carbon dioxide to force approximately the same percentage of germination at 35° C. as the lower (5 to 10) percentages of carbon dioxide did in 17 hours at 26° C. Furthermore, at this high temperature 137 hours of treatment with 60 to 80 per cent of carbon dioxide were required to obtain better than 80 per cent germination of these lettuce seeds. The effectiveness of the carbon dioxide treatment in comparison with no treatment at 35° C. is outstanding especially with variety No. 5138. In this case the data in Table III show that the maximum percentages of germination for the carbon dioxide treatment and control treatment were 99 and 1 respectively.

In reviewing the data in Table III one finds that during 17 to 41 hours of treatment at 26° C. high percentages of carbon dioxide retard the germination of lettuce seeds. However, this condition does not continue long since the high percentages of carbon dioxide will bring about the germination of these seeds within 65 hours. At the higher temperatures a directly opposite result is obtained, that is, germination takes place only in the high percentages of carbon dioxide. The reason for the low or even zero percentage germination obtained with low percentages of carbon dioxide at 30° and 35° C. is not entirely clear at this time. These ungerminated seeds are not injured since germination may be obtained by removing the seed coats, by reducing the temperature of the germinator, or by exposing the seeds to high percentages of carbon dioxide with oxygen. It is also true that those seeds remaining ungerminated in the tests exposed to high percentages of carbon dioxide are not injured and may be germinated by any of the above methods.

Black-seeded varieties. As shown by the data in Table IV the black seeds respond to the forcing action of carbon dioxide much more readily than do the white seeds. This is to be expected since the black seeds are not retarded in germination to the same extent as the white seeds with each

increase in temperature above 20° C. In general what has already been discussed in regard to the effect of time, temperature, and percentage of carbon dioxide on the white seeds is also true with the black seeds, although the effect of each factor is not as clearly shown in Table IV as

TABLE IV

GERMINATION OF BLACK-SEEDED VARIETIES OF LETTUCE SEEDS IN CARBON DIOXIDE WITH 20 PER CENT OF OXYGEN*

Variety No.	Temp. °C.	Hrs. of treatment	Percentage germination in the following percentages of carbon dioxide						
			0	5	10	20	40	60	80
5140	26	17	50	59	40	45	68	49	80
		65	74	88	49	63	94	98	97
	30	17	5	13	11	14	63	66	62
		41	8	17	18	14	68	69	91
		65	11	18	18	16	68	91	98
		113	11	18	22	20	68	95	100
	35	17	9	12	20	17	54	40	52
		41	10	15	28	18	61	72	73
		65	10	17	28	18	58	72	73
		89	22	24	29	27	63	84	83
5164	26	17	67	66	60	56	0	0	1
		65	87	82	70	78	38	92	95
	30	17	22	12	3	4	4	0	7
		41	28	14	5	5	8	28	34
		65	30	14	6	5	8	49	57
		113	30	16	6	5	17	85	96
	35	17	0	0	1	0	0	0	0
		41	0	0	1	0	0	10	15
		65	0	0	1	0	2	12	19
		89	0	0	1	0	4	68	76
5284	26	17	65	67	67	68	10	52	29
		65	92	91	95	94	92	95	96
	30	17	26	38	25	29	29	41	28
		41	39	54	57	54	75	71	52
		65	45	80	70	77	75	88	62
		113	57	83	80	83	87	94	92
	35	17	4	16	7	12	16	32	24
		41	7	18	12	22	53	61	58
		65	8	18	13	24	53	63	64
		89	8	20	13	26	61	88	97

* The seeds were exposed to considerable daylight during these tests.

in Table III. Furthermore, varietal differences in response to the carbon dioxide atmospheres are also in evidence with the black seeds.

As may be observed in Table IV the black seeds of varieties Nos. 5140 and 5284 gave, in almost every case, higher percentage germination in concentrations of carbon dioxide ranging from 5 to 80 per cent than they

did in the control lots of seeds. With these varieties of black seeds all percentages of carbon dioxide forced some germination within 17 hours at 35° C., which is to be compared with 65 hours for germination of white seeds only when held in 40 to 80 per cent of carbon dioxide at 35° C. The third variety of black seeds, No. 5164, responded more slowly to the carbon dioxide treatment at 35° C. In this case a longer period of treatment with the higher percentages of carbon dioxide was necessary to bring about germination. At germinating temperatures of 26° C. and 30° C. variety 5164 responded very well to the treatment with carbon dioxide. The variation in response of these varieties to the conditions of these experiments are very hard to explain upon the basis of harvest dates since varieties 5140 and 5164 were harvested in August and September while variety 5284 was harvested one month later.

GERMINATION IN DARKNESS IN THE PRESENCE OF CARBON DIOXIDE

Carbon dioxide is effective in forcing the germination of lettuce seeds in darkness as well as in light. As shown by the data in Table V much better germination is obtained in complete darkness when the seeds are exposed to carbon dioxide than when the seeds are held in the absence of the gas. Furthermore, a higher percentage of germination was obtained in the lots of seeds exposed to carbon dioxide in complete darkness than from similar lots of seeds held as controls in light. For example, seeds held in complete darkness at 26° C. in 80 per cent of carbon dioxide with 20 per cent of oxygen gave from 65 to 90 per cent germination while the seeds held in light without carbon dioxide at 26° C. for the same length of time showed only 25 to 54 per cent germination. In complete darkness without carbon dioxide similar lots of these seeds produced a maximum of 2 per cent germination. The spread in the germination percentages is due to differences in variety response to the germinating conditions. Lower concentrations of carbon dioxide in darkness produce a correspondingly lower percentage of germination of the lettuce seeds. Twenty per cent or less of carbon dioxide is relatively ineffective in forcing the germination of the white seeds during short periods of treatment. Should longer germinating periods be employed there would be found a corresponding increase in the percentage of germination of the seeds exposed to carbon dioxide at any of the temperatures studied.

One observes in Table V that as the temperature of the germinator is increased above 26° C. there is a decided decrease in the germination of the control seeds in light, but at the same time carbon dioxide is still effective in forcing germination in darkness. The element of time is extremely important in these experiments since any percentage of carbon dioxide that produces germination in 72 hours at 30° C. must be employed for 96 hours at 35° C. to produce the same result. In view of the data in

Table III the results in Table V would have been better if the tests had been conducted for periods as long as 140 hours instead of 96 hours. There is evidence in Table V that there is considerable variation in the response of individual varieties to the carbon dioxide treatment with time.

TABLE V

FORCING GERMINATION OF LETTUCE SEEDS IN DARKNESS WITH CARBON DIOXIDE IN THE PRESENCE OF 20 PER CENT OF OXYGEN

Variety No.	Temp. ° C.	Hrs. of treatment	Percentage germination							
			In light, control	In darkness with the following percentages of carbon dioxide						
				0	5	10	20	40	60	80
White-seeded										
5130			25	2	4	1	0	3	14	65
5138	26	48	54	0	4	4	1	43	85	86
5146			51	0	0	0	0	17	90	90
5130			1	0	1	0	0	0	1	8
5138	30	72	4	1	2	2	1	21	47	34
5146			8	0	0	2	4	4	44	22
5130		72	0	0	0	0	0	0	0	0
5130		96	0	0	0	1	0	0	1	8
5138	35	72	3	1	1	1	0	7	19	10
5138		96	4	1	3	7	6	23	66	56
5146		72	0	0	0	0	0	2	18	2
5146		96	0	0	0	2	5	5	39	33
Black-seeded										
5140			97	9	17	12	26	78	96	94
5164	26	48	97	1	7	2	2	4	13	52
5284			95	6	14	12	13	51	90	90
5140			38	11	20	18	41	65	97	68
5164	30	72	45	0	0	0	0	5	9	48
5284			89	0	2	1	9	46	65	79
5140		72	17	16	14	33	35	28	21	16
5140		96	21	22	31	41	48	78	85	86
5164	35	72	0	0	0	0	0	0	7	3
5164		96	0	0	0	0	4	4	33	46
5284		72	23	0	1	0	4	18	48	6
5284		96	24	1	1	1	6	30	80	67

The black-seeded lettuce seeds, as shown in Table V, offer comparable responses in germination to light, darkness, and to carbon dioxide as do the white-seeded varieties. High percentages of carbon dioxide forced almost complete germination of seeds of two varieties and 52 per cent germination of seeds of the third in complete darkness within 48 hours at 26° C. The control seeds in darkness gave a maximum of only 9 per cent

germination while those in light gave almost complete germination within the same period of time in the germinator. As previously mentioned there are some varietal differences in response to carbon dioxide treatment that require further investigation to work out the time relationship. It is particularly noticeable that the black seeds respond to treatment with as little as 5 per cent of carbon dioxide at 26° C.

With the increase in temperature to 30° or 35° C. the effectiveness of light in aiding germination of the black seeds diminishes even as does the germination of the control seeds in darkness. However, carbon dioxide is just as effective in forcing germination of the seeds at high as at low temperatures except that the period of treatment must be longer in the former than in the latter temperature. For example, variety No. 5164 (Table V) may be used to demonstrate this. The seeds treated with 80 per cent of carbon dioxide and 20 per cent of oxygen in darkness gave approximately the same percentage of germination in 48 hours at 26° C., in 72 hours at 30° C., and in 96 hours at 35° C. During the same periods of time the seeds held as controls in darkness gave only 1 per cent germination at 26° C. and zero percentage at the higher temperatures. In light, maximum germination was obtained only at the low temperatures, while no germination was obtained at 35° C. The other two varieties, 5140 and 5284, responded very readily to the action of carbon dioxide in forcing germination in darkness, at the various temperatures of the germinator. Of course time, temperature, and light had similar effects on the germination of these varieties as has been discussed for variety 5164.

In Tables III and IV we have observed that the lettuce seeds in light germinated readily at 26° C. in low percentages of carbon dioxide, but as the temperature was increased higher percentages of carbon dioxide and longer periods of treatment were necessary to bring about germination. A similar condition was observed with the tests in darkness except that a much lower temperature was necessary to bring about germination of the seeds in low percentages of carbon dioxide. At 20° C. in darkness the lettuce seeds germinated very well in 5 and 10 per cent of carbon dioxide but were somewhat retarded in germination by 20 per cent of carbon dioxide during the first 48 hours in the germinator. Higher percentages of carbon dioxide retarded for 48 hours the germination of all varieties except 5140 and 5284. With a period of 72 hours or longer in the germinator, seeds of the six varieties were germinating in all percentages of carbon dioxide. The control seeds germinate equally well in either light or darkness within 48 hours as is shown by the data in Table II. With the increase in temperature of the dark germinator to 26° C. or higher the seeds required high concentrations of carbon dioxide to bring about germination. These observations of the response of lettuce seeds to various conditions

for germination offer many interesting physiological problems for future research.

GERMINATION OF SEEDS ALREADY DORMANT

Lettuce seeds held under moist conditions with coats intact at 35° C. become dormant and will not germinate unless the seed coat is removed or the temperature is reduced. If, however, these dormant seeds with intact coats are exposed to high percentages of carbon dioxide with 20 per cent of oxygen at 35° C. germination will take place within a short time. The data in Table VI show the effectiveness of 40 to 80 per cent of

TABLE VI

FORCING GERMINATION AT 35° C. OF SEEDS THAT HAVE BEEN MADE DORMANT BY HOLDING UNDER GERMINATING CONDITIONS IN LIGHT AT 35° C.

Variety No.	% germ. after 96 hrs. in the presence of 20% O_2 and the following % CO_2				% germ. of same lots when removed from CO_2 atmosphere and placed in air for 48 hrs.			
	0	40	60	80	0	40	60	80
5130*	0	14	6	0	0	14	27	88
5138*	0	33	93	27	0	47	100	67
5164**	0	100	94	33	0	100	94	73
5284**	0	53	74	60	0	53	94	60

* White-seeded.
** Black-seeded.

carbon dioxide in bringing about germination of the dormant seeds of both the white- and black-seeded varieties. It is of special interest that the action of carbon dioxide is to bring about a condition within the seeds so that even if they do not germinate during the treatment they will germinate later when removed to a germinator in normal air. As shown in Table VI in almost every case there is a further increase in percentage of germination of the dormant seeds following an exposure to carbon dioxide. In no case, even with prolonged holding, did the control seeds germinate.

GROWTH OF GERMINATING SEEDS DURING AND AFTER EXPOSURE TO CARBON DIOXIDE

The growth of the radicle of lettuce seeds germinating in as high as 20 per cent of carbon dioxide was not noticeably slower or different from the growth of the untreated seeds, germination of which was obtained by removal of coats. Seedlings exposed to this amount of carbon dioxide in the germinator for as long as 96 hours developed, while in the gas, root hairs apparently as well as the control seedlings. However, there is a very marked reduction in the growth of the radicle of seeds germinating in higher percentages of carbon dioxide. For example, radicle growth of 20

mm. in the control and in as high as 20 per cent of carbon dioxide may be reduced to 2 mm. with exposure to 40 per cent and to 1 mm. or less with exposure to 60 or 80 per cent of carbon dioxide in the germinator for 65 hours. If the seeds, germinating in 60 to 80 per cent of carbon dioxide, are removed to air within 24 hours after the radicle breaks through the seed coats further growth will take place at a rate comparable with the control seeds without coats even at a temperature of 35° C. Exposure of the seeds *after germination* to the high percentages of carbon dioxide for 48 to 72 hours results in varying degrees of injury to the radicle. Slight injury is manifested by a reduced rate of growth of the radicle after removal of the carbon dioxide. In tests with long exposure, where considerable injury is apparent, the growing point is blackened and upon removal to air there are produced a few root hairs and a new main root. From this period development takes place at a normal rate. Seeds exposed to 40 to 80 per cent of carbon dioxide for 96 hours or longer after germinating are injured so that the radicle will not develop upon removal to air.

GERMINATION IN REDUCED SUPPLY OF OXYGEN

Preliminary tests were made in light at 26° and 35° C. on the rate of germination of lettuce seeds (5130 and 5164) in 10 per cent of oxygen with and without carbon dioxide. At 26° C. the results of the germination tests were comparable with those already observed in Tables III and IV. However, at 35° C. the effect of the reduced oxygen supply was very noticeable. During seven days in the germinator there were found germinated only 4 per cent of the seeds of 5164 and 1 per cent of the seeds of 5130 in a mixture of 10 per cent O_2 and 40 per cent CO_2. Germination of the seeds in the control and all other mixtures of carbon dioxide and oxygen did not take place at this high temperature. These preliminary results indicate in general the necessity of a high percentage of oxygen in the germinator as well as the presence of carbon dioxide to bring about the germination of lettuce seeds at high temperatures.

GERMINATION IN INCREASED SUPPLY OF OXYGEN

The suggestion has been made that the reason for the inability of the lettuce seed to germinate at high temperatures is due to insufficient oxygen passing through the seed coat. The solution to this problem was considered to be merely the increasing of the oxygen supply to the seeds held under the conditions found to bring about dormancy. However, the tests made upon the seeds of recently harvested white-seeded varieties (5130, 5138, 5146) do not substantiate this theory. The data in Table VII show that the lettuce seeds held at either 30° or 35° C. in as high as 90 per cent of oxygen did not germinate more readily than the control seeds in 20 per cent of oxygen at the same temperatures. This lack of response of the

seeds to increased percentages of oxygen in the atmosphere was found regardless of whether the seeds were exposed to oxygen immediately upon becoming moist or after the seeds had been held moist in the germinator at 30° or 35° C. for some time preceding the increase in the oxygen supply. These tests were conducted for a maximum of eight days with no further germination than that shown in Table VII for the four-day period. As would be expected, an increase in the oxygen supply at low temperature does hasten germination of the lettuce seeds.

TABLE VII

GERMINATION OF LETTUCE SEEDS IN VARIOUS PERCENTAGES OF OXYGEN

Variety No.	Temp. ° C.	Percentage germination in 96 hours in the following percentages of oxygen					
		20	30	40	50	70	90
White-seeded							
5130	30	2	1	2	0	0	3
	35	0	0	2	2	3	3
5138	30	4	7	4	3	3	4
	35	3	0	3	2	6	3
5146	30	0	2	0	1	1	1
	35	0	0	0	1	1	1
3099*	30	32	56	30	76	89	86
	35	1	0	1	1	3	51
Black-seeded							
5140	30	25	46	31	46	50	51
	35	20	25	26	15	31	22
5164	30	34	54	68	65	69	44
	35	1	1	1	8	13	2
5284	30	95	94	90	94	96	89
	35	4	17	31	28	42	20

* New York No. 12 seeds harvested in 1933.

The black-seeded varieties of lettuce seeds do respond to the increased supply of oxygen in the atmosphere of the germinator as is shown by the data in Table VII. However, attention must be directed to the fact that at 35° C. the increase in oxygen supply does not greatly aid, in a uniform manner, the germination of varieties Nos. 5140 and 5164 as it does variety No. 5284. This divergence in the results must be attributed to varietal differences since the harvest dates show that the seeds benefited most by the increased supply of oxygen were those most recently harvested.

These results on the effect of increasing the oxygen supply to lettuce

seeds at high temperatures are not entirely in accord with the general conclusions that Borthwick and Robbins (1) drew from their experiments with seeds of a New York variety of lettuce. A few tests were made upon this variety of lettuce, seeds of which were harvested in 1933 and since held in dry storage at 25° to 30° C. Although the germination results of the New York variety shown in Table VII are not entirely comparable with their results with the freshly-harvested seeds the data show that at 30° C. an increase in oxygen supply will aid the germination of seeds of this variety. However, at 35° C. even with these old seeds, concentrations of oxygen less than 70 per cent in the germinator did not bring about an increase in the germination of the seeds. The data show that at least from 70 to 90 per cent of oxygen is necessary to produce approximately 50 per cent germination of the seeds of this variety of lettuce. This lack of complete response by these lettuce seeds is not due to seeds of poor vitality since germination tests made at 20° C. gave an average of 97 per cent germination within 48 hours.

The germination of the recently harvested lettuce seeds held in increased partial pressures of oxygen took place in the majority of the tests with the growth of the radicle followed by the growth of the cotyledons as is usually the case with germinating seeds. In a few cases, occasionally in the controls as well as in the oxygen treatments, some of the lettuce seeds germinated with the protrusion of the cotyledons through the distal end of the seed coats. The growth of the cotyledons was slowly followed by the elongation of the hypocotyl and growth of the radicle. Low percentage germination of this type was found consistently only with varieties Nos. 5130, 5140, and 5164. This type of germination has been reported (1) to occur completely with the New York variety of lettuce when the seeds are exposed to high oxygen pressures at 30° C. Tests made upon the 2-year-old seeds of this variety show that most of the germination in oxygen does take place with the primary growth of the cotyledons. This is another marked difference in response in germination of lettuce seeds that must be attributed to varietal differences and should be investigated more completely with seeds of the same relative age.

DISCUSSION

These findings that germination of lettuce seeds can be induced by treatment with carbon dioxide are in contrast to those of Kidd (4, 5) who obtained inhibition of the germination of seeds of barley, pea, bean, cabbage, onion, and white mustard by exposing them to carbon dioxide. This treatment even induced dormancy in the white mustard seeds. The situation with recently harvested lettuce seeds is quite different since carbon dioxide does not inhibit germination or induce dormancy, but on the

contrary causes the prompt germination of the seeds under conditions that are distinctly unfavorable in the absence of carbon dioxide. Previous results with cocklebur (9) also are against the view that Kidd's finding of an inhibitive effect of carbon dioxide is of general application.

Possibly the germination of non-dormant seeds (such as were used mainly by Kidd) is unfavorably affected by carbon dioxide, but if so it is interesting that in the case of at least two seeds in the dormant condition (lettuce and cocklebur) germination is distinctly favored by this same agent.

In these experiments carbon dioxide has been used as a substitute for certain temperature and light conditions that previously have been considered necessary for germination of freshly-harvested lettuce seeds. Thus, seeds that would germinate in darkness only if given temperatures of 20° C. or lower, germinated successfully at temperatures as high as 35° C. if treated with carbon dioxide, and seeds that required an exposure to light first, and even then needed a temperature of 26° C. or less, were induced to germinate in darkness at 35° C. by exposure to carbon dioxide. Whatever the effects of light and of the low temperatures upon lettuce seeds, these may be replaced by an exposure to carbon dioxide.

The means by which carbon dioxide induces germination may not be the same as those by which light and temperature operate but the observable effects upon the percentage of germination are the same.

SUMMARY

Heretofore temperatures below 20° C. in darkness, and below 26° C. in light, have been considered to be necessary for good germination of recently harvested lettuce seeds.

The experiments here reported show that in the presence of carbon dioxide good germination may be obtained even at 35° C., either in light or in darkness.

At 20° to 26° C. from 5 to 20 per cent of carbon dioxide with 20 per cent of oxygen will bring about germination of the lettuce seeds within 17 hours. With an increase in temperature of the germinator to 35° C. higher percentages (from 40 to 80 per cent) of carbon dioxide and approximately 65 hours of treatment are needed to produce germination of the seeds.

Lettuce seeds made dormant by moist storage at 35° C. in the absence of carbon dioxide will germinate at this same temperature during and after exposure to mixtures of approximately 40 to 80 per cent of carbon dioxide and 20 per cent of oxygen for 96 hours.

Lettuce seeds that have been forced to germinate by the use of carbon dioxide produce good seedlings which grow well when subsequently transferred to air.

LITERATURE CITED

1. BORTHWICK, H. A., and W. W. ROBBINS. Lettuce seed and its germination. Hilgardia **3**: 275–304. 1928.
2. DAVIS, W. E. The germination of lettuce seed. Proc. Assoc. Off. Seed Anal. N. Amer. **16**: 71–73. 1924.
3. FLINT, LEWIS H. Light in relation to dormancy and germination in lettuce seed. Science **80**: 38–40. 1934.
4. KIDD, FRANKLIN. The controlling influence of carbon dioxide in the maturation, dormancy, and germination of seeds. Part I. Proc. Roy. Soc. [Lond.] B. **87**: 408–421. 1914.
5. ——— The controlling influence of carbon dioxide in the maturation, dormancy, and germination of seeds. Part II. Proc. Roy. Soc. [Lond.] B. **87**: 609–625. 1914.
6. ROSE, DEAN H. A study of delayed germination in economic seeds. Bot. Gaz. **59**: 425–444. 1915.
7. SHUCK, A. L. Some factors influencing the germination of lettuce seed in seed laboratory practice. New York [Geneva] Agric. Exp. Sta. Tech. Bull. 222. 21 pp. 1934.
8. ——— The formation of a growth inhibiting substance in germinating lettuce seeds. Proc. Internat. Seed Test. Assoc. **7**: 9–14. 1935.
9. THORNTON, NORWOOD C. Factors influencing germination and development of dormancy in cocklebur seeds. Contrib. Boyce Thompson Inst. **7**: 477–496. 1935.

INDUCED CHANGES IN RESPIRATION RATES AND TIME RELATIONS IN THE CHANGES IN INTERNAL FACTORS[1]

LAWRENCE P. MILLER, JOHN D. GUTHRIE, AND F. E. DENNY

Among the internal factors that have been suggested as influencing, or even controlling, the rate of respiration of plant tissue, the following have received considerable attention: sugar concentration (especially that of sucrose); activity of catalase and peroxidase; methylene blue reducing capacity; hydrogen ion concentration; and non-enzymic catalysts of cellular oxidation, such as glutathione.

In some of these cases various workers have reported good correlation between the respiration and the factor studied, in that high or low oxidation corresponded to high or low values of the factor. Usually the importance upon respiration of only one or two factors was studied in any one experiment and the time relation between the increase or decrease in the carbon dioxide production and the increase or decrease in each factor studied was not established.

In previous experiments (13, 14) it was found that the CO_2 output of potato tubers could be increased 300 to 600 per cent by treating them with vapors of ethylene chlorhydrin (3), and could be decreased 50 to 90 per cent by treating them with vapors of ethyl alcohol. The increase with ethylene chlorhydrin began about ten hours, and the decrease with alcohol about three hours, after the start of the treatments. A means is thus afforded by which large differences in respiration can be produced at will and a study can be made of the correlation in the changes in the various factors and the change in respiration, not only as to the amount and direction of the change, but also as to the time after treatment at which these changes became manifest.

In this paper are reported the results of experiments in which potato tubers were subjected to the vapor of various chemicals which induced changes in the CO_2 output, and analyses for various constituents were made at different time intervals after the start of the treatments. In this way it was possible to determine not only what changes were closely associated with changes in respiratory activity but also in what relative order these changes occurred. Experiments were conducted with ethylene chlorhydrin, ethyl alcohol, ethyl mercaptan, hydrocyanic acid, butyl chloride, butyl bromide, and butyl iodide. Changes in CO_2 output, sugar, glutathione, pH, sulphuric acid, citric acid, catalase and peroxidase activity were studied, all these determinations being made on the same sample.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 107.

MATERIALS AND METHODS

The experiments here reported were performed with dormant potato tubers (*Solanum tuberosum* L.), variety Irish Cobbler. Potatoes from several sources were employed. Some were obtained from the Institute gardens, others from a second crop grown in the late summer in New Jersey, and still others from South Carolina and from Maine. The same general results were obtained irrespective of the lot of tubers used.

The chemicals used for the treatments were chosen because previous experiments had indicated that they would offer excellent means of studying the effect of various factors supposedly important in influencing or regulating respiration. Thus, ethylene chlorhydrin was known to markedly increase and ethyl alcohol to markedly decrease the CO_2 output. Ethyl mercaptan had been found to increase respiration and to decrease catalase and peroxidase activity (12) and hydrocyanic acid increased respiration but in contrast to ethylene chlorhydrin produced less of an increase in the glutathione content (8). The butyl halides had been found to increase respiration markedly (15) but no study had been made of any other changes taking place as a result of treatment with these chemicals.

The tests were carried out as follows. A quantity of tubers weighing from 1000 to 2500 grams, depending upon the number of samples that were to be taken for analysis, was put into a desiccator of seven and one-half liter capacity which was immersed in a thermostatically-controlled water bath. A measured amount of chemical was put on a piece of cheesecloth and placed in the closed desiccator for the desired period, usually about 24 hours, after which a stream of air was drawn through the desiccator and then through Van Slyke-Cullen tubes (18) containing $Ba(OH)_2$ of known strength to absorb the CO_2 given off (13). At any time that a sample of tubers was desired for analysis the stream of air was interrupted for a short time and some of the tubers removed. The determination of the CO_2 output of the tubers remaining in the desiccator was continued as soon as possible after the removal of the sample and a continuous record of the CO_2 output was obtained until the last sampling, when all the remaining tubers were used for the analysis. In the case of the experiments with ethyl alcohol and ethylene chlorhydrin the chemical was furnished to the tubers with the stream of air being drawn through for the CO_2-output determinations and it was thus possible to sample the tubers before the end of the treatment period. The results then showed how soon after the start of the exposure to the chemical the respiratory change set in (14) and also what time relationship the other changes had with the increase or decrease in CO_2 output resulting from treatments with ethylene chlorhydrin and ethyl alcohol respectively. From one to three different treatments and a control were run at the same time.

The various determinations made on the tubers removed from the con-

tainers at intervals after the start of the treatments were made on samples of about 500 grams. For such of the determinations as were made on whole tissue, pieces were cut from each of the tubers and combined to make a representative portion of the whole sample. The material remaining was then put through a food grinder and the juice squeezed through cheesecloth and centrifuged. The juice thus obtained was then used for the analyses which were to be made on the juices. In the case of some of the experiments (Table II) certain analyses were made on tissue which had been preserved in alcohol at the time of sampling.

Further details of the method used for the determination of the CO_2 output can be obtained from previous papers (12, 13). From the methods used it follows that the data given for the rate of CO_2 output at various intervals after treatment do not represent instantaneous rates but are the average rates for a number of hours previous to the time of sampling. When the time between two sampling periods was long, more than one rate determination was made during the interval, but in each case the determination immediately preceding the time of sampling is, of course, the one given in the tables.

Catalase activity was determined by measuring the O_2 liberated from H_2O_2 with the use of the apparatus and procedure described by Davis (2) except that the H_2O_2 was neutralized with $CaCO_3$ instead of NaOH. Peroxidase was measured by the amount of purpurogallin formed from pyrogallol by a modification of Willstätter's method (12, p. 34). For the determination of reducing sugars and sucrose the copper reduction was carried out under the conditions described by Quisumbing and Thomas (16) and the amount of cuprous oxide formed was determined by titration with potassium permanganate after solution with sulphuric acid containing ferric ammonium sulphate. Sucrose was hydrolyzed to invert sugar by invertase prepared from yeast (17). The methylene blue reducing capacity of the juice was determined in the same manner as in previous experiments (5).

The pH determinations were made on the expressed juice using the quinhydrone electrode. Although determinations were made on the juice directly after expressing from the tissue, the values reported are those obtained after removal of the carbon dioxide from the juice by areation with nitrogen. In some cases the juice was boiled and filtered before aeration with nitrogen. The removal of the carbon dioxide from the juice gave more consistent values, since a variable loss of carbon dioxide from the juice was unavoidable during expressing and handling. By completely removing the carbon dioxide, this variable was eliminated. The pH differences obtained by this procedure with ethylene chlorhydrin and other chemicals that increase the respiration are larger than obtained with unaerated juice, since the treated juice contains more carbon dioxide than the untreated juice.

TABLE I

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENTS ON POTATO TUBERS*

Determination	Tr. and ck.	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5			
		Hours from start of treatment											
		16.5	40.8	21.7	45.4	12.6	60.5	12.3	61.5	5.0	23.3	47.6	71.6
Mg. CO_2 per 100 g. per hr.	Tr.	10.24	15.88	9.52	15.35	3.66	8.34	1.36	5.42	1.10	2.98	6.60	4.85
	Ck.	2.08	1.97	2.52	2.21	2.09	1.66	1.05	1.01	1.01	1.24	1.12	0.99
Mg. CO_2 to sampling time	Tr.	169	554	140	504	45	553	17	249	5	48	208	328
	Ck.	33	81	51	103	26	114	13	67	5	27	50	78
pH aerated expressed juice	Tr.	6.76	7.51	6.73	7.20	6.12	7.24	6.03	6.88	5.93	6.15	6.68	6.86
	Ck.	5.92	6.14	6.02	5.90	6.07	5.95	6.03	6.07	5.97	6.03	6.07	6.09
Citric acid, mg. per 100 cc.	Tr.	293	222	332	205	437	238	523	335	517	471	352	313
	Ck.	387	383	451	470	470	448	484	524	517	475	479	513
Glutathione mg. per 100 g.	Tr.	16.4	39.2	11.0	35.8	8.0	26.0	10.6	28.4	9.8	9.0	18.2	23.0
	Ck.	11.4	13.0	11.2	11.6	8.0	11.4	10.4	11.0	10.2	10.6	12.4	10.8
SO_4, cc. N/10 H_2SO_4 per 100 cc.	Tr.	10.6	7.9	10.8	8.5	9.6	7.7	14.1	12.5	12.4	12.1	12.7	11.8
	Ck.	11.3	10.5	11.6	9.4	12.0	11.1	12.0	14.6	13.7	13.5	13.2	13.9
Total sugars mg. dextrose per 5 cc.	Tr.			13.7	50.0	19.1	56.7	15.6	42.0	22.9	16.3	24.6	38.7
	Ck.			20.1	20.9	17.7	20.7	19.2	18.0	15.3	21.4	19.7	20.3

* Treatments were made by adding chlorhydrin vapor to stream of air being drawn through containers for CO_2 output determination (14). Time of exposure to vapor: Experiment 1, 24 hours; Experiment 2, 21.7 hours; Experiment 3, 23.1 hours; Experiments 4 and 5, 24 hours.

Citric acid was determined by the official pentabromacetone method of Hartmann and Hillig (11) using in most cases 50 cc. of the boiled, filtered juice. These values are reported in terms of mg. citric acid per 100 cc. In some of the experiments, the determinations were made on dried tissue that had been previously killed in alcohol. These values are reported in terms of mg. of citric acid per 100 g.

Glutathione was determined by the sulphur reduction method (10) that has been employed in previous experiments. Since it was found that under the conditions of the determinations, the yield of hydrogen sulphide from added glutathione was 50 per cent of the theoretical value required by the equation for the reaction of sulphur with glutathione, this was taken into consideration in the calculation of the glutathione values.

Sulphate was determined by precipitation as barium sulphate from the boiled, filtered juice after acidification with hydrochloric acid. After the first ignition of the barium sulphate it was digested with hydrochloric acid, washed with water, and again ignited.

RESULTS

The data obtained are tabulated in the accompanying tables. Some of the relationships found are shown graphically in Figures 1 to 5.

Sugars. Previous conclusions (4, 13) on the effect of chemical treatments on the sugar content and on the relation of changes in sucrose to the changes in the respiratory rate are confirmed by the present experiments. Chemical treatments which increase the CO_2 output usually cause an increase in the sucrose content but this increase does not occur until after the rise in respiration; in fact, the sugar content may decrease during the period of rapid rise of the CO_2 output. This is brought out clearly in the data of Table III in which samples taken from 24 to 47.4 hours after the start of treatment show little or no increase in the sucrose content although CO_2 output has increased several hundred per cent. In the case of the butyl iodide treatments a decrease in the sugar content is shown by the first samples although the respiratory rate is several times that of the controls. Large increases in the sucrose content are evident at later sampling periods. The data show definitely that there is no increase in sucrose at the time of the rise in the CO_2 output and that the large increases do not occur until the time when the respiratory rate is dropping. The data are thus completely at variance with the hypothesis that the concentration of sucrose controls the respiratory rate (1). Decreases in the sugar content were obtained in the treatments with ethyl mercaptan although these produced large increases in respiration; treatments with ethyl alcohol had only small effects on the sugar content but decreases were frequently evident.

Catalase and peroxidase. The results of the catalase and peroxidase de-

terminations confirm and extend those reported in previous papers (5, 7, 12). In the present paper the determinations were made on the same samples which had been used for the determination of respiratory activity and thus are more valid for direct comparisons. Also in the case of the butyl halides, catalase and peroxidase values are here given for the first time. The data show that there is no direct connection between the respiratory rate and catalase and peroxidase activity. In those treatments in which the activity of these enzymes increases as a result of exposure to the chemical the increase takes place later than the start of the increase

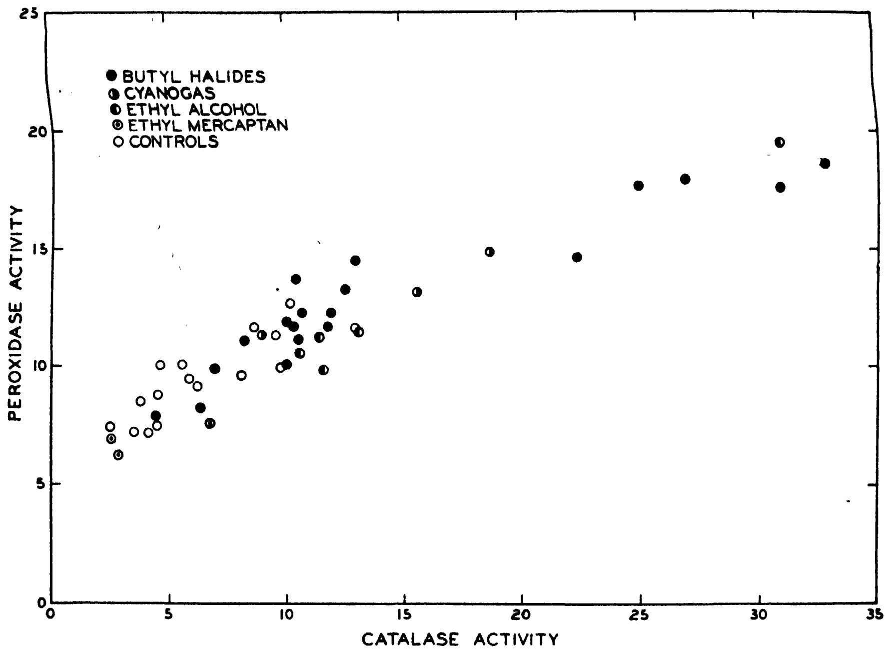


FIGURE 1. Correlation between peroxidase activity and catalase activity in control tubers and tubers treated with various chemicals.

in CO_2 output; it is only after the peak of the respiratory rise has almost passed that these enzymes become most active. With ethyl mercaptan the catalase and peroxidase activity are actually depressed much below the control while the rise in CO_2 output is taking place and during its peak; it is only later that these enzymes show greater activity in the treated sample than in the check. Alcohol which decreases respiration causes an increase in catalase activity and only very small changes in peroxidase activity which may become somewhat lower than the control (7).

It is of interest to note that with the methods here employed there is a close correlation between catalase activity and peroxidase activity in

that when one changes the other also usually changes in the same direction and to a similar extent. The only exception to this close connection is ethyl alcohol which may show an increase in catalase and at the same time a decrease in peroxidase. All determinations of catalase and peroxidase activity on the tubers obtained from South Carolina, including samples at various intervals after treatment, and controls as well as tubers treated with the butyl halides, cyanogas, ethyl mercaptan, and ethyl alcohol, are shown in the scatter diagram in Figure 1. The close correlation between these two enzymes under such a variety of conditions is very striking.

The tubers used for the experiments reported in Table II were from a different source and the determinations of enzyme activity were made on samples of thoroughly ground tissue instead of juice. But here too a good correlation between the two enzymes is evident in the ethylene chlorhydrin treatments. In the treatments with alcohol, however, the two enzymes diverge, one increasing and the other decreasing.

Methylene blue reducing capacity of the juice. As previously reported (5, 7) an increase was noted with ethylene chlorhydrin treatments. However, it will be seen from Table II that the increase in methylene blue reducing capacity took place later than the increase in respiration. The treatments with butyl bromide and butyl iodide, which are reported in Table IV, also increased the methylene reducing capacity of the juice. In these cases also, the increase took place after the increase in respiration had begun.

Effect of high respiration on the citric acid content and on the hydrogen ion concentration. The fact that ethylene chlorhydrin produces large changes in various constituents of the tissues makes possible the study of these changes in relation to each other with the object of gaining some insight into the chemical reactions that go on in the tissue. In order to show a relationship between such changes, there are several criteria that should be considered. One is whether the relation is chemically reasonable from both the qualitative and quantitative standpoint, the second is that the time relations should fit in with our hypothesis concerning the changes, and the third is that it should be possible to show a correlation between the changes. The results of the citric acid and pH determinations offer support for the idea that these values are greatly influenced by the respiration of the tissue. It appears that citric acid is one of the substrates for respiration in the potato tuber and that it is converted to carbon dioxide in the rapidly respiring, ethylene chlorhydrin-treated tissue. This results in a decrease in citric acid and consequently the juice contains less acid and is therefore more alkaline. The following discussion of the results will show how nearly the criteria given above have been met in regard to the relationship between respiration, citric acid, and pH.

The effect of various chemicals on the respiration, citric acid, and pH

EFFECT OF TREATMENT WITH ETHYLENE CHLORHYDRIN AND ETHYL ALCOHOL ON POTATO TUBERS*

Determination	Treatment	Experiment 1: Hours from start of treatment					Experiment 2: Hours from start of treatment				
		15.8	39.8	63.8	87.0	159.8	11.3	16.9	35.7	59.8	108.0
Mg. CO_2 per 100 g. per hour	Chlorhydrin	3.74	8.78	9.49	7.44	2.64	1.44	3.82	6.32	7.75	3.50
	Alcohol	1.40	1.67	1.95	2.07	1.50	0.62	0.86	1.21	1.16	1.54
	Control	2.36	2.06	2.46	2.15	2.78	1.05	1.61	1.36	1.24	1.30
Mg. CO_2 per 100 g. up to time of sample	Chlorhydrin	59	268	494	667	877	16	37	156	343	512
	Alcohol	22	63	109	157	266	7	12	35	63	137
	Control	37	87	146	196	408	12	21	47	76	149
pH of expressed juice after aeration with N	Chlorhydrin	6.24	6.81	6.86	6.91	6.83	6.07	6.20	6.34	6.76	6.70
	Alcohol	5.80	5.85	5.92	5.76	—	6.09	6.07	6.09	6.00	6.09
	Control	5.93	5.93	5.97	5.88	5.95	6.09	6.10	6.09	6.09	6.19
Citric acid mg. per 100 g.	Chlorhydrin	350	326	368**	310	291	442	410	368	386	343
	Alcohol	462	403	387**	412	428	425	444	439	384	441
	Control	408	448	466**	467	461	442	444	450	415	384
Catalase, cc. O_2 by 0.1 g. in one minute	Chlorhydrin	7.9	14.3	20.3	21.6	11.6	9.6	10.0	10.2	17.0	13.4
	Alcohol	9.0	9.3	13.7	13.1	14.4	8.1	10.3	8.8	8.9	13.3
	Control	8.1	7.4	8.9	7.5	6.0	9.2	8.3	6.6	7.2	5.0
Peroxidase, mg. purpurogallin per gram	Chlorhydrin	3.8	11.1	18.6	15.2	11.3	5.1	5.9	5.6	8.6	10.9
	Alcohol	4.6	4.6	6.7	4.3	5.5	4.0	5.4	3.6	2.8	4.3
	Control	3.9	5.4	9.4	5.7	5.5	5.1	6.0	5.1	4.2	4.8
Glutathione mg. per 100 g.†	Chlorhydrin	5.4	6.2	21.6	29.0	33.6	8.2	9.4	10.4	22.2	27.0
	Alcohol	5.8	6.0	5.8	8.4	—	8.8	7.6	8.2	6.6	9.8
	Control	6.4	5.4	4.8	5.2	5.2	8.4	8.2	7.0	6.4	6.4
Reducing sugars % of dry weight	Chlorhydrin	2.01	1.67	0.07**	0.94	1.11	1.04	1.46	1.57	1.18	1.45
	Alcohol	1.15	1.46	1.21**	1.51	1.26	1.12	1.43	1.24	1.58	1.30
	Control	1.44	1.50	0.68**	1.06	0.99	1.17	1.60	1.52	1.50	1.47
Sucrose % of dry weight	Chlorhydrin	1.14	1.31	2.79	3.66	3.42	1.12	1.11	1.53	2.21	2.77
	Alcohol	1.43	1.08	1.08	1.10	1.46	0.98	0.93	1.04	0.95	1.25
	Control	1.18	1.16	1.15	1.20	1.31	1.05	1.06	1.07	1.09	1.06
Methylene blue reduction‡	Chlorhydrin						>60	>60	>60	12	25
	Alcohol						>60	>60	>60	>60	>60
	Control						>60	>60	>60	>60	>60

* Chemicals were furnished to tubers in stream of air being drawn through for the CO_2 output determinations. Time of exposure, 24 hours.
** These samples were heated too much while drying. † Corrected for recovery. ‡ Time in minutes for reduction.

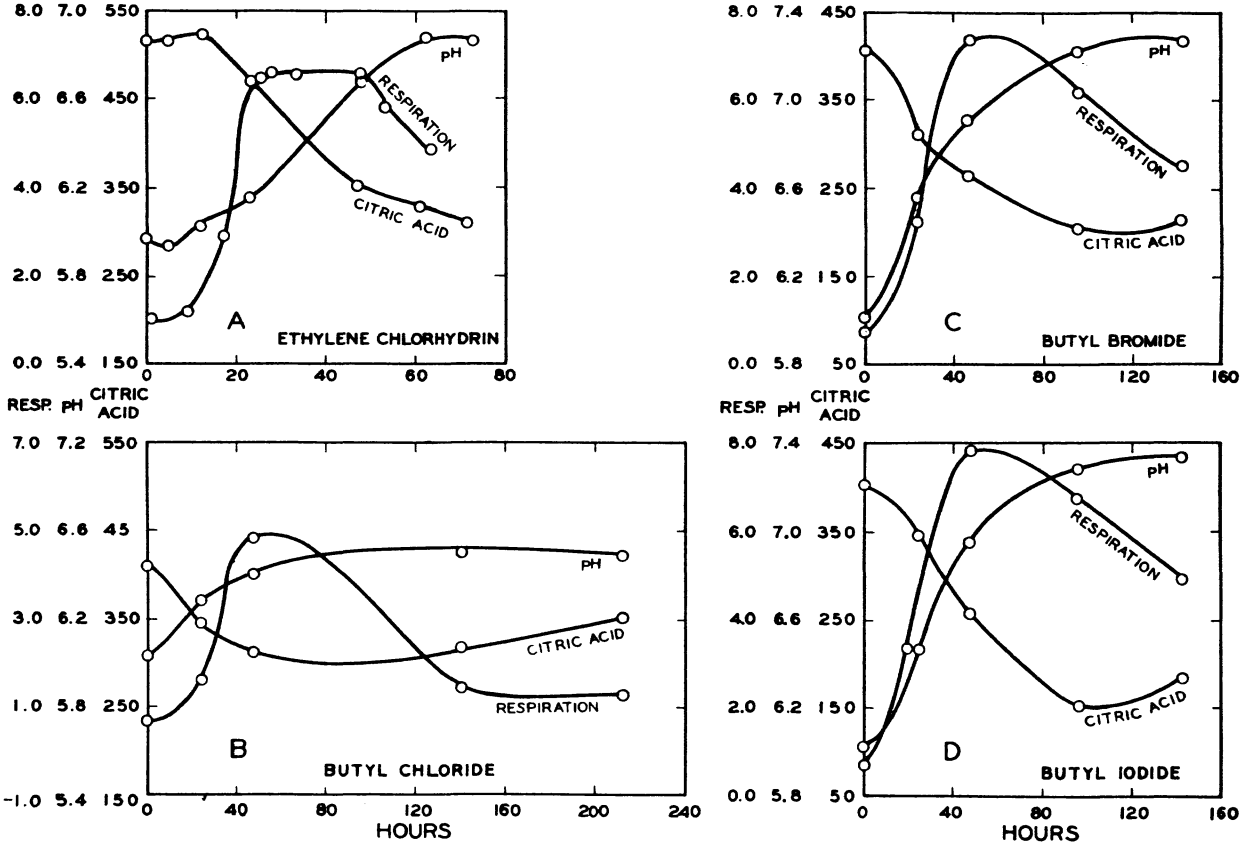


FIGURE 2. Relation between respiration, citric acid, and pH in potato tubers treated with ethylene chlorhydrin and with butyl halides.

TABLE III

EFFECT OF TREATMENTS WITH THE BUTYL HALIDES ON POTATO TUBERS*

Determination made	Experiment 1					Experiment 2				
	Halide used	Hours from start of treatment				Halide used	Hours from start of treatment			
		23.9	47.4	142.4	215.2		24.0	47.0	95.9	143.4
Mg. CO_2 per 100 g. per hour	Chloride	1.61	4.84	1.46	1.25					
	Bromide	2.16	6.35	4.13	3.32	Bromide	3.32	7.47	6.13	4.52
	Iodide	1.90	4.97	1.39	1.58	Iodide	3.38	7.94	6.80	4.97
	Control	0.71	0.95	1.22	1.44	Control	0.84	1.48	1.06	0.74
Mg. CO_2 per 100 g. up to sampling time	Chloride	38.5	132.0	329.9	420.7					
	Bromide	51.6	172.6	668.9	914.7	Bromide	79.7	258.7	591.3	803.3
	Iodide	45.4	142.8	333.2	441.8	Iodide	81.0	271.4	625.7	859.5
	Control	16.9	37.0	148.7	243.7	Control	19.3	53.6	110.8	145.4
pH of boiled aerated juice	Chloride	6.27	6.39	6.49	6.46					
	Bromide	6.47	—	6.90	6.71	Bromide	6.56	6.90	7.08	7.25
	Iodide	6.54	6.93	6.64	6.59	Iodide	6.47	6.96	7.29	7.34
	Control	6.17	6.12	6.14	6.17	Control	6.12	6.10	6.19	6.22
Citric acid mg. per 100 cc. juice	Chloride	345	310	324	348					
	Bromide	307	—	238	270	Bromide	310	266	202	214
	Iodide	305	298	315	316	Iodide	346	258	154	188
	Control	363	417	420	415	Control	402	400	339	412
Catalase activity cc. O_2 per 0.8 cc. juice in 2 min.	Chloride	4.4	10.4	10.2	8.2					
	Bromide	6.9	—	30.8	22.2	Bromide	12.4	9.9	11.8	32.7
	Iodide	6.3	24.8	10.6	9.9	Iodide	10.3	11.7	12.8	26.8
	Control	3.5	3.7	2.5	4.1	Control	4.6	4.5	5.8	4.5

* Quantities of chemicals used were as follows: Experiment 1, butyl chloride, 0.3 cc. per l.; butyl bromide, 0.1 cc. per l.; butyl iodide, 0.05 cc. per l.; tubers exposed to chemicals for 22.25 hours; Experiment 2, butyl bromide, 0.15 cc. per l.; butyl iodide, 0.10 cc. per l.; time 22 hours.

TABLE III (*Continued*)

Determination made	Experiment 1					Experiment 2				
	Halide used	Hours from start of treatment				Halide used	Hours from start of treatment			
		23.9	47.4	142.4	215.2		24.0	47.0	95.9	143.4
Peroxidase activity, mg. purpurogallin per 1.6 cc. juice	Chloride	7.8	11.0	11.6	11.0					
	Bromide	9.8	—	17.6	14.6	Bromide	13.2	10.0	12.2	18.6
	Iodide	8.2	17.6	12.2	11.8	Iodide	13.6	11.6	14.4	17.8
	Control	7.2	8.4	7.4	6.9	Control	10.0	7.4	9.4	8.8
Glutathione mg. per 100 cc. (corrected for recovery)	Chloride	4.5	9.5	12.2	13.5					
	Bromide	1.0	1.0	9.9	13.9	Bromide	0.5	1.0	1.0	6.9
	Iodide	4.5	10.3	13.8	16.7	Iodide	2.0	1.5	1.0	5.1
	Control	12.7	12.3	8.6	10.5	Control	11.7	11.5	8.1	7.4
Sulphate cc. N/10 per 100 cc. juice	Chloride	7.2	7.1	7.4	7.7					
	Bromide	6.1	—	7.4	6.6	Bromide	6.5	5.6	5.0	7.0
	Iodide	6.1	6.6	7.4	7.3	Iodide	6.1	5.4	5.4	5.6
	Control	7.5	7.6	8.2	8.1	Control	7.2	7.1	5.7	6.6
Reducing sugars mg. per 4 cc. juice	Chloride	0.8	0.8	1.6	0.8					
	Bromide	0	—	2.9	2.5	Bromide	0	0	0	3.4
	Iodide	0	0.9	1.7	0.5	Iodide	0	0	0	2.3
	Control	0	0	1.5	0.8	Control	0	0	0	0
Total sugars mg. per 4 cc. juice	Chloride	15.4	16.7	27.5	25.5					
	Bromide	10.7	—	49.7	54.4	Bromide	15.4	22.6	24.9	53.2
	Iodide	7.9	17.0	28.0	24.8	Iodide	5.3	21.4	44.1	46.4
	Control	17.1	14.7	17.9	20.1	Control	12.6	13.4	12.5	12.3
Methylene blue reduction, time in minutes	Chloride	>60	>60	>60	>60					
	Bromide	>60	—	1	15	Bromide	>60	10	60	10
	Iodide	>60	7	60	30	Iodide	>60	10	10	10
	Control	>60	>60	>60	>60	Control	>60	>60	>60	>60

are shown in Tables I to V. It will be seen that with ethylene chlorhydrin treatments the decrease in citric acid and the increase in pH occurred at about the same time as the increase in respiration. As shown in Table I, experiment 1, the respiration had increased greatly and the citric acid had decreased as early as 16.5 hours after the beginning of treatment. An increase in pH had also taken place. In experiment 2 these changes were noted in the first sample taken 21.7 hours after the beginning of treatment. Since experiments 4 and 5 were run on the same material at nearly the same time, they may be considered together. For this purpose the results have been plotted in Figure 2 A. The first points plotted for citric acid and pH are the initial control values. The other points are the values for the treated samples. Additional respiration values that have not been included in the table have been added. It will be seen that the decrease in citric acid and the increase in pH began at the same time that the rise in respiration occurred. Additional evidence that the decrease in citric is simultaneous with the increase in respiration is given in Table II, experiment 2. There was no change in citric acid and very little change in respiration 11.3 hours after the beginning of treatment, but respiration increased and citric decreased 16.9 hours after treatment.

Butyl chloride, butyl bromide, and butyl iodide treatments greatly increased the respiration, decreased the citric acid content, and increased the pH. The results of analyses made on tissue treated with these chemicals are given in Table III. It will be seen that so far as respiration and citric acid were concerned, the results were the same as with ethylene chlorhydrin. In all cases, an increase in respiration, a decrease in citric acid, and an increase in pH had occurred at the first sampling period, 24 hours after the beginning of the treatment. Some of these results are plotted in Figure 2, B, C, and D. In so far as the data permit it will be seen that the decrease in citric acid occurred at the same time as the increase in respiration.

The effect of the ethyl alcohol treatments was to decrease the respiration of the tissue. The results of ethyl alcohol treatments are given in Tables II, IV, and V. The change in citric acid was small and of rather doubtful significance, but in some cases a decrease occurred. It is interesting to note that the effect of the alcohol was to produce a more acid juice. Since no increase in citric acid occurred, this decrease in pH must be due to an increase in some undetermined acid. The decrease in pH observed in these experiments with ethyl alcohol treatments is at variance with previous results (7) where an increase in pH was found. The explanation for this is that the tubers were not cut up and planted, but were kept in the respiration chambers until the conclusion of the experiment. In previous experiments the tubers were cut up immediately after treatment and planted in soil. The effect of the subsequent treatment of the tissue on

TABLE IV

EFFECT OF HYDROGEN CYANIDE AND OF ETHYL ALCOHOL ON POTATO TUBERS*

Determination	Experiment 1					Experiment 2				
	Treat-ment	Hours from start of treatment				Treat-ment	Hours from start of treatment			
		24.8	47.0	95.9	144.5		27.2	48.8	71.6	120.2
Mg. CO_2 per 100 g. per hour	HCN	1.91	4.24	2.39	1.83					
	Alcohol	0.61	1.45	2.61	1.80	HCN	2.58	4.14	2.92	2.16
	Control	1.18	2.04	1.49	1.62	Control	1.42	2.30	2.04	1.56
Mg. CO_2 per 100 g. up to sampling time	HCN	47	143	272	361					
	Alcohol	15	48	169	256	HCN	70	160	226	331
	Control	29	75	165	244	Control	39	89	135	211
pH, boiled aerated juice	HCN	6.22	6.34	6.34	6.36					
	Alcohol	6.07	6.14	6.00	6.41	HCN	6.22	6.27	6.29	6.32
	Control	6.22	6.20	6.14	6.19	Control	6.29	6.29	6.24	6.25
Citric acid, mg. per 100 cc. juice	HCN	355	350	324	344					
	Alcohol	357	336	335	325	HCN	658	574	528	530
	Control	340	355	404	346	Control	617	585	591	572
Catalase, cc. O_2 per 0.8 cc. juice in 2 minutes	HCN	8.9	18.5	27.0	15.5					
	Alcohol	10.4	11.4	13.0	30.8					
	Control	9.7	9.5	6.2	5.5					
Peroxidase, mg. purpurogallin per 1.6 cc. juice	HCN	11.2	14.8	—	13.0					
	Alcohol	0.5	9.8	11.4	19.4					
	Control	19.9	11.2	9.2	10.0					
Glutathione, mg. per 100 g.	HCN	8.7	11.4	15.6	15.0					
	Alcohol	10.4	10.8	12.6	14.3	HCN	9.4	9.0	10.8	11.4
	Control	10.0	11.8	10.7	12.3	Control	9.6	11.0	9.4	9.6
SO_4, cc. N/10 H_2SO_4 per 100 cc. juice	HCN	6.6	6.1	6.1	6.3					
	Alcohol	5.8	7.9	5.7	6.1	HCN	12.6	10.8	8.8	12.3
	Control	7.2	6.6	7.7	7.2	Control	11.5	11.5	10.4	11.8
Reducing sugars mg. per 4 cc. juice	HCN	0	1.7	6.0	1.6					
	Alcohol	0	0	0	0 2					
	Control	0.8	1.4	0	1.					
Total sugars, mg. dextrose per 4 cc. juice	HCN	11.2	19.6	33.5	22.9					
	Alcohol	15.7	8.9	12.1	17.9	HCN	25.2	27.6	28.4	—
	Control	16.1	19.1	15.1	22.5	Control	28.4	24.8	30.0	—
Methylene blue reduction, time in minutes	HCN	>60	>60	>60	>60					
	Alcohol	>60	>60	>60	>60					
	Control	>60	>60	>60	>60					

* Quantities of chemicals used were as follows: Experiment 1 (duration of exposure 22.8 hours) hydrogen cyanide, 0.1 g. cyanogas per l.; ethyl alcohol, 1.75 cc. per l.; Experiment 2, 0.1 g. cyanogas per l. for 24 hours.

the response to ethyl alcohol has been verified in an experiment on the same lot of tubers.

The results of treatment with ethyl mercaptan are given in Table V. Ethyl mercaptan increased the respiration and brought about a decrease in citric acid. However, the pH picture is complicated by the fact that the first effect of the mercaptan treatments was to make the juice more acid. This acidification must have been due to an increase in some acid other than citric. Later this acidification was overcome and the treated sample became more alkaline than the control.

The results of treatments with hydrogen cyanide are shown in Table IV. The changes produced by these treatments were small, but a small increase in respiration took place, and in experiment 2, this was accompanied by a slight decrease in citric acid and a slight increase in pH.

If the above interpretation of the results is correct, there should be a correlation between the change in respiration, the change in citric acid, and the change in pH. The correlation between the change in citric acid and the change in respiration in terms of total carbon dioxide produced is shown in Figure 3. In this diagram the changes in citric acid are plotted against the corresponding changes in total carbon dioxide production. These values were obtained by finding the difference between the value for the treated sample and the value for the control sample. Consequently, both positive and negative values can occur. All of the data have been used, including each chemical tried and the samples taken at different time intervals. The alcohol treatments are somewhat exceptional, inasmuch as they show a slight decrease in citric acid in most cases, accompanied by a decrease in respiration. Aside from this, however, the diagram shows that in most cases an increase in total carbon dioxide output is accompanied by a decrease in citric acid. If our methods were perfect and citric acid were the only substance respired, we would expect the points to fall on the line that has been drawn. This line represents the theoretical yield of carbon dioxide from the complete combustion of citric acid. Part of the deviations from this line are due to errors in analysis and to variability of the tubers, but the larger discrepancies are probably due to the fact that other substances are respired in addition to citric acid. There is a tendency for the citric acid changes to be larger than expected in the cases in which the respiration change is small and smaller than expected when the respiration change is large. Of course the small respiration changes represent mostly analyses made soon after treatment, or chemicals whose effect on respiration was small. A study of the data for the chemicals producing large changes in respiration, that is, ethylene chlorhydrin and the butyl halides, shows that the ratio of extra CO_2 formed to citric acid lost is less than the theory of 1.37 for periods up to 24 hours after the

start of the treatments. This ratio, however, tends to increase, until it becomes much greater than the theory at about the 80 hour period.

On the above point, an experiment carried out on a 1000 g. sample, where the variation of the tubers consequently played a smaller part, is of interest. At the period of sampling, 95 hours after the beginning of the treatment, the loss in citric acid was 1.95 g. This contained 0.7 g. of carbon. In the same period the sample had lost 5.2 g. of carbon dioxide in

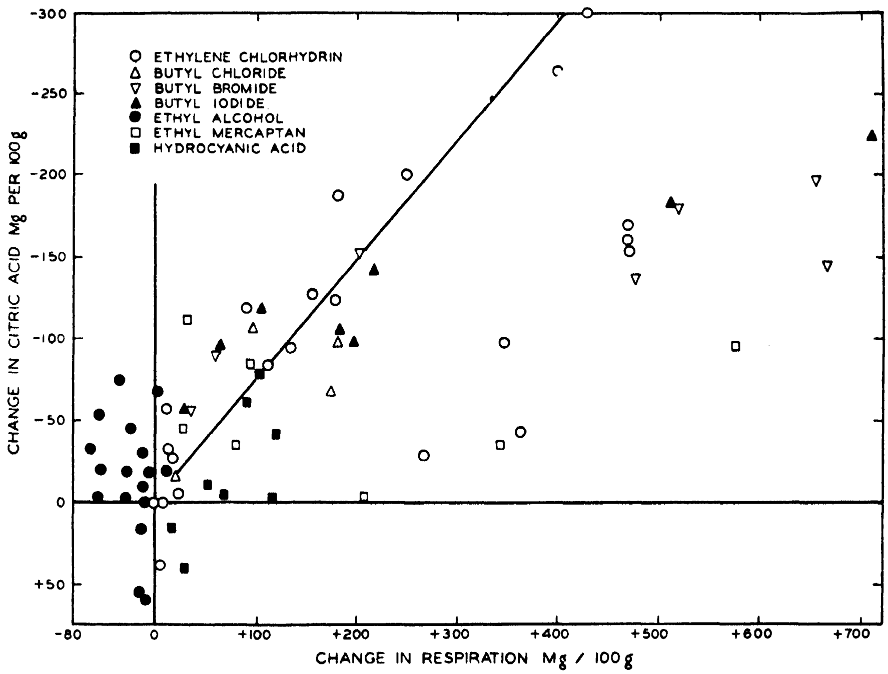


FIGURE 3. Correlation diagram in which change in citric acid content is plotted against change in respiration as measured by total carbon dioxide production. The line represents the theoretical yield of carbon dioxide from the complete combustion of citric acid.

excess of the control sample. This contained 1.4 g. of carbon. In this case, therefore, the citric acid lost from the tissue will account for one-half of the increase in carbon dioxide output. That more carbon dioxide is obtained than can be accounted for by citric acid shows that some of the carbon dioxide comes from the carbohydrates. Upon the question whether or not the respiratory process must necessarily pass through citric acid the experiments throw no light, but it is conceivable that citric acid is an intermediate compound in the breakdown of sugars to carbon dioxide.

The relation of the pH change to the change in citric acid has been dealt with in a previous paper (9) in which it was shown that the decrease

in citric acid played the most important part in the increase in pH that followed treatment of potato tubers with ethylene chlorhydrin. A correlation is, therefore, to be expected between the change in pH and the change in citric acid. In Figure 4 the change in citric acid is plotted against the change in pH, using the data for all the chemicals used in the present experiments. The correlation is good with the exception of the ethyl mercaptan values. As pointed out previously, the results with ethyl mercaptan are probably complicated by an increase in some undetermined acid.

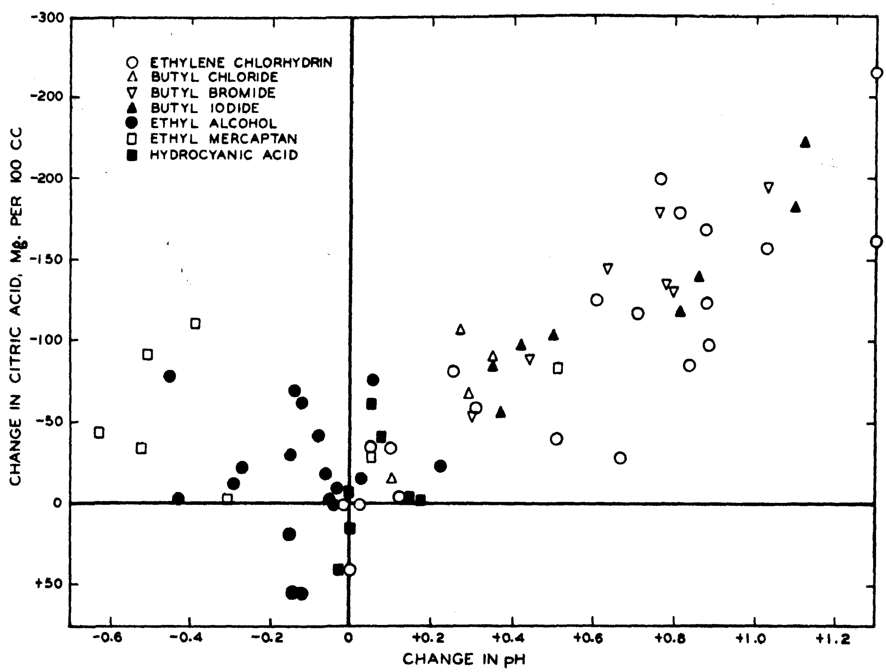


FIGURE 4. Correlation diagram in which change in citric acid content is plotted against the change in pH of the juice.

Correlation should also be shown between the change in respiration and the change in pH. This is shown in Figure 5, in which the change in pH is plotted against the change in total carbon dioxide output. Again the ethyl mercaptan values are exceptional, but some indication of a relationship is shown even here. The pH of the ethyl mercaptan samples with a high respiration is higher than those with a low respiration.

Glutathione. When glutathione was first discovered it was thought that it would be found to play an important role in the oxidation-reduction processes of cells. So far, however, experimental evidence has not established just what part glutathione plays in these processes and has been of

a rather negative nature. This is true of our results concerning glutathione and respiration. As has been shown previously (8) ethylene chlorhydrin treatments bring about a large increase in glutathione. However, the present experiments show that the increase in glutathione takes place after the increase in respiration, and consequently cannot be the cause of the increased respiration. For example, in Table I, experiment 2, a large increase in respiration had taken place at the 21.7 hour period, while no change in glutathione was found. Also, in Table I, experiment 5, at the 20 hour period respiration had begun to increase in the treated sample,

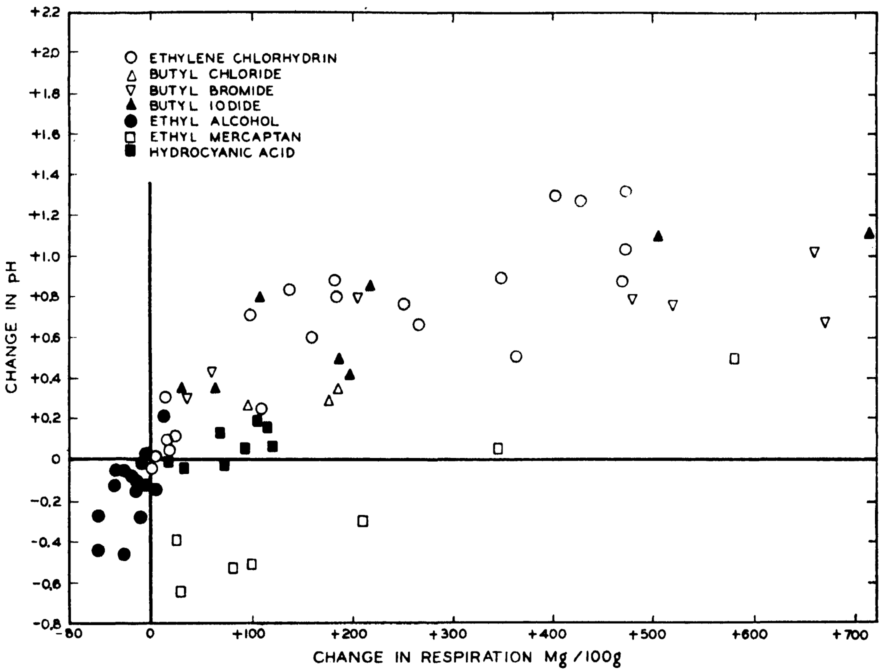


FIGURE 5. Correlation diagram in which change in pH of the juice is plotted against change in respiration as measured by total carbon dioxide production.

while no increase in glutathione was noted. In Table II, experiment 1, a large increase in respiration had taken place 39.8 hours after treatment with ethylene chlorhydrin, while at this time there was no change in the glutathione content. The glutathione change occurs at a relatively late period after the chlorhydrin treatment, the data indicating that the increase takes place 40 to 60 hours after the start of the treatment.

Treatments with ethyl alcohol increased the glutathione content slightly. These increases were not as large as those obtained in previous experiments (8). This is due to the fact that the potatoes were not cut into pieces and planted as was done in earlier experiments, but were kept

TABLE V

EFFECT OF ETHYL MERCAPTAN AND OF ETHYL ALCOHOL ON POTATO TUBERS*

Determination	Experiment 1				Experiment 2					Experiment 3			
	Treatment	Hours from start of treatment			Treatment	Hours from start of treatment				Treatment	Hours from start of treatment		
		24.3	46.4	94.5		24.0	45.8	71.0	142.8		20.4	45.0	68.8
Mg. CO_2 per 100 g. per hour	C_2H_5SH C_2H_5OH Control	2.68 0.94 1.38	5.76 1.96 2.66	7.04 ** 1.80	C_2H_5SH Control	2.49 1.20	4.51 2.24	6.49 1.39	5.68 1.43	C_2H_5OH Control	0.28 1.67	0.42 1.38	1.19 1.32
Mg. CO_2 per 100 g. up to time of sampling	C_2H_5SH C_2H_5OH Control	65 23 34	192 66 93	525 ** 179	C_2H_5SH Control	60 29	158 78	321 113	798 218	C_2H_5OH Control	6 34	16 68	44 99
pH of boiled aerated juice	C_2H_5SH C_2H_5OH Control	5.71 6.05 6.34	5.71 5.76 6.22	6.12 ** 6.05	C_2H_5SH Control	6.00 6.39	5.83 6.36	6.10 6.41	6.88 6.37	C_2H_5OH Control	5.98 6.03	5.73 6.00	5.59 6.02
Citric acid mg. per 100 cc. juice	C_2H_5SH C_2H_5OH Control	325 358 370	299 305 385	331 ** 365	C_5H_2SH Control	524 637	550 585	536 537	470 565	C_2H_5OH Control	431 433	440 461	457 459
Catalase, cc. O_2 per 0.8 cc. juice in 2 minutes	C_2H_5SH C_2H_5OH Control	2.8 11.3 12.8	2.5 8.1 10.1	6.7 ** 8.6	C_2H_5SH Control	5.2 13.3	3.0 10.8	4.0 12.1	29.9 4.7				
Peroxidase, mg. purpurogallin per 1.6 cc. juice	C_2H_5SH C_2H_5OH Control	6.2 11.2 11.6	6.9 9.6 12.6	7.6 ** 11.6	C_2H_5SH Control	13.7 17.6	8.4 17.7	9.4 18.9	36.8 13.6				
Glutathione, mg. per 100 cc. juice	C_2H_5SH C_2H_5OH Control	13.5 10.7 11.2	10.1 9.1 13.0	13.5 ** 11.2	C_2H_5SH Control	25.0 12.4	17.4 12.4	17.4 13.0	21.6 10.6	C_2H_5OH Control	11.2 14.0	10.2 12.6	8.2 11.4
SO_4, cc. N/10 H_2SO_4 per 100 cc. juice	C_2H_5SH C_2H_5OH Control	7.4 6.4 6.7	6.8 7.6 6.9	8.1 ** 5.9	C_2H_5SH Control	12.5 11.7	10.8 10.1	11.0 11.1	12.1 11.0	C_2H_5OH Control	12.6 11.2	12.0 11.3	15.0 14.1
Total sugars, mg. dextrose per 4 cc. juice	C_2H_5SH C_2H_5OH Control	2.1 18.2 19.8	0.2 22.1 16.7	0.4 ** 23.4	C_2H_5SH Control	16.3 23.2	— 26.0	0.8 25.5	19.2 31.1	C_2H_5OH Control	12.5 17.1	9.2 16.9	8.3 14.8

* Quantities of chemicals used were as follows: Experiment 1 (time of exposure 23 hours), ethyl mercaptan, 0.45 cc. per l.; ethyl alcohol, 2.75 cc. per l.; Experiment 2, ethyl mercaptan, 0.45 cc. per l. for 22.1 hours; Experiment 3, ethyl alcohol, vapor added in stream of air drawn through desiccator for CO_2 output determination; time of exposure 24 hours.

** Tissue showed injury at this stage and therefore these determinations were not made.

in the respiration chambers until the end of the experiment. As discussed above, this factor also changes the pH response. Treatment with HCN also brought about a small increase in glutathione as shown in Table IV.

The effect of the butyl halides is shown in Table III. The initial effect on the glutathione content is a marked decrease. This is observed immediately after treatment. It seems likely that this change was due to some direct action of these halogen compounds on glutathione, such as has been observed for iodoacetic acid and similar compounds (6). The depression of the glutathione content by the butyl halide treatments persisted for about four days and then an increase began, so that after six days the treated samples contained as much glutathione as the controls. The samples taken nine days after treatment showed that the treated tissue contained more glutathione than the controls.

The fact that the butyl halides decrease the glutathione content, while in other respects their effect is very similar to ethylene chlorhydrin, is of interest in connection with the view that the increase in glutathione may play a part in the breaking of dormancy by non-sulphur chemicals (8). Previous results (15) indicate that the butyl halides are less effective in breaking the dormancy than ethylene chlorhydrin, but further experiments comparing these chemicals on the same lot of potatoes would be desirable.

The results of the ethyl mercaptan treatments are complicated by the fact that ethyl mercaptan reacts like glutathione in the method for estimating the glutathione content. The high values in the treated samples immediately after treatment were therefore due to the presence of ethyl mercaptan in the tissue. The mercaptan disappears rapidly from the tissue, however, as indicated by the odor, which is a very sensitive test for the presence of ethyl mercaptan. Therefore, the values obtained at the time of the last sampling probably represent glutathione and show that ethyl mercaptan treatments increase the glutathione content.

Sulphate. As reported previously (9) treatment of potato tubers with ethylene chlorhydrin brought about a decrease in the sulphate content of the expressed juice. It is probable that this decrease is due to the utilization of sulphate sulphur in the synthesis of glutathione by the tissue. The results of the present experiments, because of the rather small samples available, are somewhat too variable to draw a definite conclusion as to just when the sulphate decrease took place, but the data are not inconsistent with the view that the sulphate decreases at the same time that the glutathione increases. Considering the data given in Table I, the average change in the sulphate content before the glutathione increase is -0.8 cc. of N/10 while the average change after the glutathione increase is -1.9 cc. N/10. There is no evidence that the chemicals used in these experiments, other than ethylene chlorhydrin, produced a change in the sulphate content.

SUMMARY

1. Potato tubers were subjected to the vapor of various chemicals under conditions which were known either to increase or decrease the CO_2 output and the time relations were determined in the changes resulting in respiration, pH, citric acid, glutathione, sugars, sulphate, catalase activity, peroxidase activity, and methylene blue reducing power.

2. Treatments which increased the CO_2 output also usually caused large increases in the sugar content but these increases did not occur until after the peak of the higher respiratory activity had passed. During the period of rapid rise of the CO_2 output, the sugar content either remained unchanged or decreased. Ethyl alcohol which decreased the CO_2 output either had little effect on the sugar content or brought about decreases.

3. Catalase and peroxidase activity also were not directly connected with the changes in CO_2 output. The chemicals which increased respiration brought about large increases in the activity of these enzymes but a study of the time relations shows that these changes tend to follow rather than precede or coincide with the changes in the CO_2 output. With ethyl mercaptan the activity of these enzymes was depressed much below the control while the rise in CO_2 output was taking place and during its peak; it was only later when the respiratory rate was decreasing that these enzymes were more active in the treated sample than in the control. Ethyl alcohol which decreased respiration increased catalase activity and decreased peroxidase activity.

4. The increase in the methylene blue reducing capacity of the juice that follows ethylene chlorhydrin or butyl halide treatments is not the cause of the increased respiration produced by these treatments, since the increase in reducing capacity takes place later than the increase in respiration.

5. The decrease in citric acid and the increase in pH begin at approximately the same time as the increase in respiration. There is a correlation between these three changes. These results are offered in support of the view that citric acid is converted to carbon dioxide in the rapidly respiring tissue and that the pH increases as a consequence of the decrease in citric acid. The data show that citric acid is probably the source of a large part of the carbon dioxide produced by the treated tissue.

6. The large increase in glutathione that follows treatment with ethylene chlorhydrin is not the cause of the increase in respiration, since it takes place 40 to 60 hours after the beginning of treatment, much later than the increase in respiration. Butyl halides, which produce large increases in respiration, decrease the glutathione content, possibly by some direct action on glutathione.

7. The decrease in sulphate that follows treatment with ethylene chlorhydrin probably occurs at about the same time as the increase in

glutathione and may be due to the utilization of sulphate in the synthesis of glutathione.

LITERATURE CITED

1. BARKER, J. The substrate of respiration in the potato. Great Britain Dept. Sci. & Indus. Res., Food Invest. Bd. Rept. **1933**: 67–69. 1934.
2. DAVIS, WILMER E. The use of catalase as a means of determining the viability of seeds. Proc. Assoc. Off. Seed Anal. N. Amer. **18**: 33–39. 1926. (*Also in* Boyce Thompson Inst. Prof. **1** (2): 6–12. 1926.)
3. DENNY, F. E. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 386–396. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 169–180. 1926.)
4. ——— Chemical changes induced in potato tubers by treatments that break the rest period. Amer. Jour. Bot. **16**: 326–337. 1929. (*Also in* Contrib. Boyce Thompson Inst. **2**: 131–142. 1929.)
5. DENNY, F. E., LAWRENCE P. MILLER, and JOHN D. GUTHRIE. Enzym activities of juices from potatoes treated with chemicals that break the rest period. Amer. Jour. Bot. **17**: 483–509. 1930. (*Also in* Contrib. Boyce Thompson Inst. **2**: 417–443. 1930.)
6. GODDARD, DAVID ROCKWELL, and MAXWELL PHILIP SCHUBERT. The action of iodoethyl alcohol on thiol compounds and on proteins. Biochem. Jour. **29**: 1009–1011. 1935.
7. GUTHRIE, JOHN D. The effect of various chemical treatments of dormant potato tubers on the peroxidase, catalase, pH, and reducing properties of the expressed juice. Contrib. Boyce Thompson Inst. **3**: 499–507. 1931.
8. ——— Change in the glutathione content of potato tubers treated with chemicals that break the rest period. Contrib. Boyce Thompson Inst. **5**: 331–350. 1933.
9. ——— Metabolism of citric, sulphuric, and nitric acid in the potato tuber. An explanation for the high pH of the juice of tubers treated with ethylene chlorhydrin. Contrib. Boyce Thompson Inst. **6**: 247–268. 1934.
10. GUTHRIE, JOHN D., and FRANK WILCOXON. Estimation of sulphydryl in tissues. Contrib. Boyce Thompson Inst. **4**: 99–106. 1932.
11. HARTMANN, B. G., and F. HILLIG. Determination of citric acid in fruits and fruit products. Jour. Assoc. off. Agric. Chem. **13**: 99–103. 1930.
12. MILLER, LAWRENCE P. Effect of sulphur compounds in breaking the dormancy of potato tubers and in inducing changes in the enzyme activities of the treated tubers. Contrib. Boyce Thompson Inst. **5**: 29–81. 1933.
13. ——— Effect of various chemicals on the sugar content, respiratory rate, and dormancy of potato tubers. Contrib. Boyce Thompson Inst. **5**: 213–234. 1933.
14. ——— Time relations in effect of ethylene chlorhydrin in increasing and of ethyl alcohol in decreasing the respiration of potato tubers. Contrib. Boyce Thompson Inst. **6**: 123–128. 1934.
15. ——— The effect of the alkyl halides on the respiration of potato tubers. Contrib. Boyce Thompson Inst. **6**: 279–296. 1934.
16. QUISUMBING, F. A., and A. W. THOMAS. Conditions affecting the quantitative deterination of reducing sugars by Fehling solution. Elimination of certain errors involved current in methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
17. REYNOLDS, F. W. The rapid analysis of sugars. Purification and concentration of enzyme solutions. Indus. & Eng. Chem. **16**: 169–172. 1924.
18. VAN SLYKE, DONALD D., and GLENN E. CULLEN. A permanent preparation of urease, and its use in the determination of urea. Jour. Biol. Chem. **19**: 211–228. 1914.

EFFECT OF GROWTH SUBSTANCES ON THE ROOTING RESPONSE OF CUTTINGS

A. E. HITCHCOCK AND P. W. ZIMMERMAN

Previous reports from this laboratory concerning the effect of synthetic growth substances on plants (2, 3, 4, 5, 7, 8) have dealt primarily with the treatment of intact plants. The present paper deals with the influence of these substances on the rooting response of cuttings, particularly cuttings of woody species. Since in some cases very pronounced favorable effects were obtained, it would appear that the proper use of certain of the several effective compounds now known will no doubt be of considerable practical value in propagating many species of plants which are either difficult to root or which require long periods to form roots. Although lanolin preparations of the growth substances have been used to induce many types of formative responses on herbaceous plants, water solutions of the same substances were found to be much more effective in causing root formation in cuttings, particularly in those made from the woody species of plants.

MATERIALS AND METHODS

The following crystalline acids were used: indoleacetic and indolebutyric obtained from R. H. Manske of the National Research Council, Ottawa, Canada; indolepropionic and phenylacetic purchased from the Eastman Kodak Company; and naphthaleneacetic which was made in our laboratory.

The growth substances were either dissolved in tap water or mixed with lanolin. A detailed description of the methods and procedure for each type of preparation appears under separate headings.

EXPERIMENTAL RESULTS

WATER PREPARATIONS OF THE GROWTH SUBSTANCES

Solutions of each growth substance were prepared by first dissolving the crystals in a few drops of 95 per cent ethyl alcohol and then adding the required amount of tap water. The low solubility of these substances in water made such a procedure necessary in order to obtain rapid solution. Dilutions were made from a standard stock solution containing, as a rule, 20 mg. per 100 cc. of water. Fresh stock solutions were made each day. Tap water containing an equivalent amount of alcohol (0.2 to 0.5 per cent) was used as a control solution. This amount of alcohol neither stimulated root formation nor caused any injurious effects on the cuttings as evidenced by comparative tests with tap water minus the alcohol. Dilutions

made from the stock solution contained correspondingly lower percentages of alcohol.

In most tests the basal ends of the cuttings were placed in minimum amounts of the test solution which usually represented a column one-half to three-fourths of an inch in height, depending upon the diameter of the container and the size of the cuttings. After remaining in the test solution for the required period, the cuttings were planted in the rooting medium in the greenhouse together with control lots which had remained in tap water for the same period. In a few special tests the cuttings were inverted during treatment and were then placed in an upright position, and completely buried in the rooting medium.

All cuttings were planted and cared for according to the methods previously described (6). Briefly, this method consists of leaving some or all leaves on the lower part of the cutting and then so placing the cutting in the rooting mixture that from two-thirds to seven-eighths of the stem is buried and the leaves or parts of leaves exposed lie close to or nearly flat on the surface of the mixture which consists of half peat moss and half sand, by volume. Cheesecloth is generally placed on the cuttings during the first ten days or three weeks, or during the entire time the cuttings are left in the rooting mixture, depending upon the species of cutting and the atmospheric conditions prevailing at the time. The cheesecloth cover was left on all holly cuttings, described in this paper, during the entire five or six weeks they remained in the rooting mixture, except during periods of continuous cloudy weather when the cover was removed.

In general, hardwood cuttings without leaves responded to the treatments much less readily than hardwood cuttings with leaves. Since tests with water solutions of the growth substances were begun in the fall, most of these results apply to woody species which were dormant and hence the cuttings were made from mature wood. Due to the fact that many treatments were highly effective on cuttings of the American holly, and since the greatest number of treatments were applied to this species, a detailed description of the results will be given only for this series of experiments.

Ilex opaca Ait. Cuttings of the American holly were taken during the last week in December, most of them on the 30th and 31st days. The cuttings were made from six-year-old plants growing on the Institute grounds. Large root systems were obtained in five to six weeks' time as a result of treatment with solutions of either indoleacetic acid or naphthaleneacetic acid (Figs. 1 and 2). These compounds were effective over a range in concentration of from 10 to 20 times, when the duration of the treatment was from 6 hours to 4 days. The highest effective concentration for 6 to 18 hours was usually toxic to cuttings left in the same solutions for 54 to 96 hours. Low concentrations that were effective for the 54- to 96-hour periods were not effective at the shorter periods of 24 hours or less.

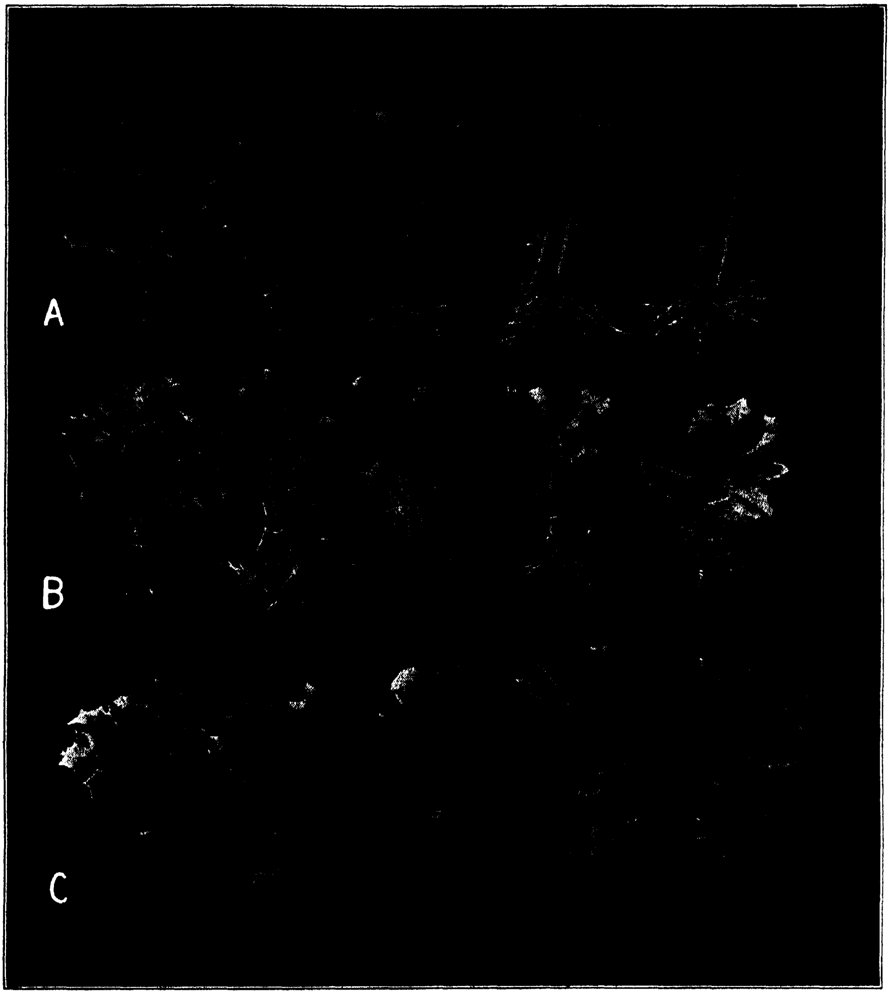


FIGURE 1. Rooting response after 5 weeks (Dec. 30 to Feb. 4) of *Ilex opaca* cuttings treated with solutions of growth substances. A, 6-hour treatment with indoleacetic acid, left to right: tap water control, 4, 10, 20, and 40 mg. per 100 cc. B, 54-hour treatment with same concentrations of indoleacetic acid. C, naphthaleneacetic acid, from left to right: tap water 4 days, 1 mg. per 100 cc. for 2 days, 1 mg. 4 days, 2 mg. 4 days, and 20 mg. 6 hours. Controls root only at base of cuttings. Cuttings planted in peat moss-sand mixture after solution treatment.

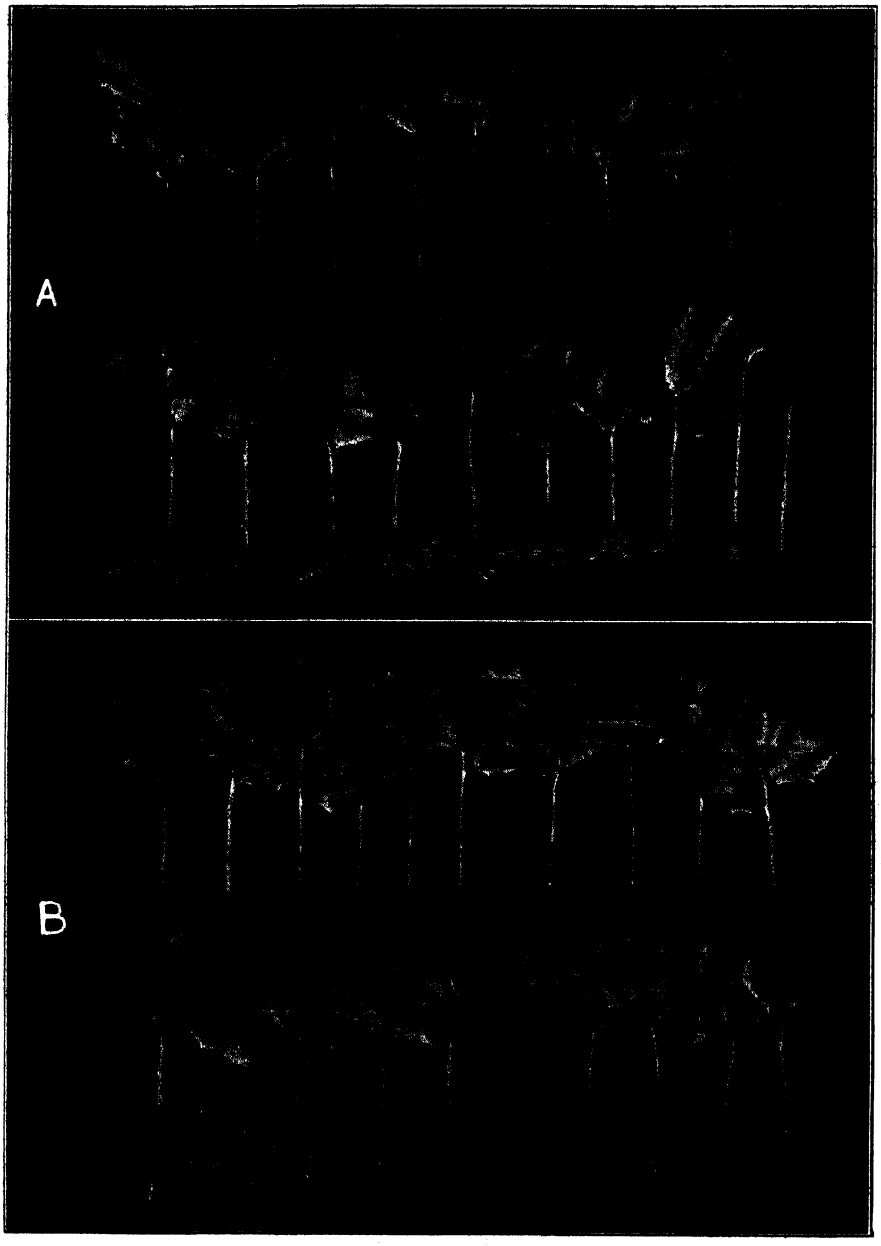


FIGURE 2. Rooting response after 5 weeks of *Ilex opaca* cuttings. A, basal ends in tap water (top row) for 54 hours, and in indoleacetic acid, 10 mg. per 100 cc. (lower row) for 54 hours. B, similar treatment with naphthaleneacetic acid (lower row) 2 mg. for 4 days. Top row, controls.

The most effective treatments were as follows: for indoleacetic acid—40 mg. per 100 cc. for 6 to 8 hours, 20 mg. for 6 to 48 hours, 10 mg. for 18 to 54 hours, and 4 mg. for 54 hours; for naphthaleneacetic acid—20 mg. for 6 hours, 10 mg. for 6 to 24 hours, 4 mg. for 6 to 24 hours, 2 mg. for 24 to 48 hours, and 1 mg. for 54 to 96 hours.

The effectiveness of the treatments was exhibited in a number of ways—by much earlier rooting, more roots, a larger root system, growth of roots from stem tissue above the base as well as at the base, and a close relationship between rooting response, concentration of growth substance, and the duration of the period of treatment. These relationships are illustrated in Figure 1. The root growth induced by two of the most effective treatments is shown in Figure 2. Non-treated tap water control cuttings were just beginning to root at the time these photographs were taken. Roots appeared only from the basal ends of control cuttings.

Naphthaleneacetic acid was effective in slightly lower concentrations than indoleacetic acid. On the other hand, indoleacetic acid was effective over longer periods of treatment than equivalent concentrations of naphthaleneacetic acid. If the duration of the treatment were extended beyond the optimum time for any given concentration, naphthaleneacetic acid caused injurious effects such as are shown in Figure 1 B (lot of cuttings on the right) at shorter time periods than was the case for indoleacetic acid.

Indolepropionic acid was much less effective than either indoleacetic or naphthaleneacetic acids. In fact, the former was effective only at or near the point where injury occurred. For example, 40 mg. per 100 cc. for 18 hours was the only indolepropionic acid treatment which was effective at the end of five to six weeks. Indolebutyric acid was not available at the time these tests were made, so that this compound was not used in treating *Ilex opaca* cuttings.

Ilex crenata Thunb. Cuttings of the Japanese holly responded in a similar manner to treatments with naphthaleneacetic acid and indolebutyric acid. As a result of treatment with either of these compounds, large root systems were obtained in three to four weeks. Indolebutyric acid was approximately five times more effective than naphthaleneacetic acid. The optimum treatment for indolebutyric acid was 0.4 to 2 mg. per 100 cc. for 16 to 24 hours, and for naphthaleneacetic acid 2 to 5 mg. for 16 to 24 hours. In this particular series of experiments indoleacetic acid was not used.

In some of the tests cuttings were made of two-year wood with one-year shoots attached to the upper portion. After the basal end of the two-year wood had remained in the test solution for the required time, the one-year-old shoots from the upper portion were removed and planted in the rooting mixture together with the treated two-year-old cuttings. High concentrations of naphthaleneacetic acid which caused roots to emerge all along

the stems of the two-year-old cuttings, also induced roots to emerge all along the stems of the one-year-old shoots, indicating that the growth substance had moved into the one-year-old shoots in a sufficient amount during treatment to induce root growth from regions of the stem where roots are not normally produced. Cuttings treated similarly with tap water produced roots only from the basal ends. The treatment induced root growth earlier and caused the formation of many more roots as compared to the response of the tap water controls.

Pachysandra terminalis Sieb. & Zucc. Cuttings of *Pachysandra* taken during November and December and treated with solutions of naphthaleneacetic acid showed increased root growth due to the treatment after having remained in tap water or in the peat moss-sand mixture for four weeks. Normally (on control cuttings) roots emerge from the axil of buds near the base of the cutting. In the case of the most effective treatments, however, the roots emerged in rows from internodal tissue as well as from the nodes. The most effective treatments were 2 to 10 mg. per 100 cc. for 1 day, 0.4 to 5 mg. for 3 days, and 0.1 to 2 mg. for 6 to 9 days. These concentrations induced many roots to grow from tissue above the base and up along the stem for distances of one to three inches. High concentrations retarded bud growth.

The emergence of many roots through bark tissue above the basal cut surface occurred also as a result of treating dormant cuttings of *Hibiscus syriacus* L. with solutions of indolebutyric acid (0.8 to 4 mg. per 100 cc. for 3 days) and softwood cuttings of *Cytisus maderensis* Masf. with solutions of indoleacetic acid (4 mg. per 100 cc. for 24 hours). In the case of *Cytisus*, the softwood cuttings had rooted in 14 days, but cuttings made from mature shoots and given similar treatment had not rooted in this time.

Syringa vulgaris L. Root growth on young shoots of the lilac was increased by treatment with solutions of naphthaleneacetic acid. From 1 to 2 mg. per 100 cc. of tap water for 24 hours appeared to be one of the most effective treatments. In many of the treated lots roots emerged from stem tissue above the base as well as at the base. Treatments which proved slightly toxic caused roots to grow on some of the cuttings from all regions of the stem up to the terminal bud. The shoots used in these experiments were forced to grow as a result of treatment of large potted plants with vapors of ethylene chlorhydrin during November 1935. Treatment of these shoots with solutions of naphthaleneacetic acid was carried out during December.

Taxus cuspidata Sieb. & Zucc. Earlier rooting of *Taxus* cuttings was obtained by treatment with naphthaleneacetic acid. The rooting response shown on the treated lot in Figure 3 resulted from treatment with 4 mg. per 100 cc. for 24 hours. Higher concentrations caused more roots to

emerge from stem tissue above the base, but at least some of the cuttings given this treatment were injured. Treatment of terminal cuttings having from four to six side shoots caused roots to emerge from the basal end and also from stem tissue above. In this case the roots emerged mainly from the region where side shoots were attached to the main stem. On control cuttings roots appeared from only the basal end of the main stem.

Special tests. Treatment of several varieties of cuttings with dilute

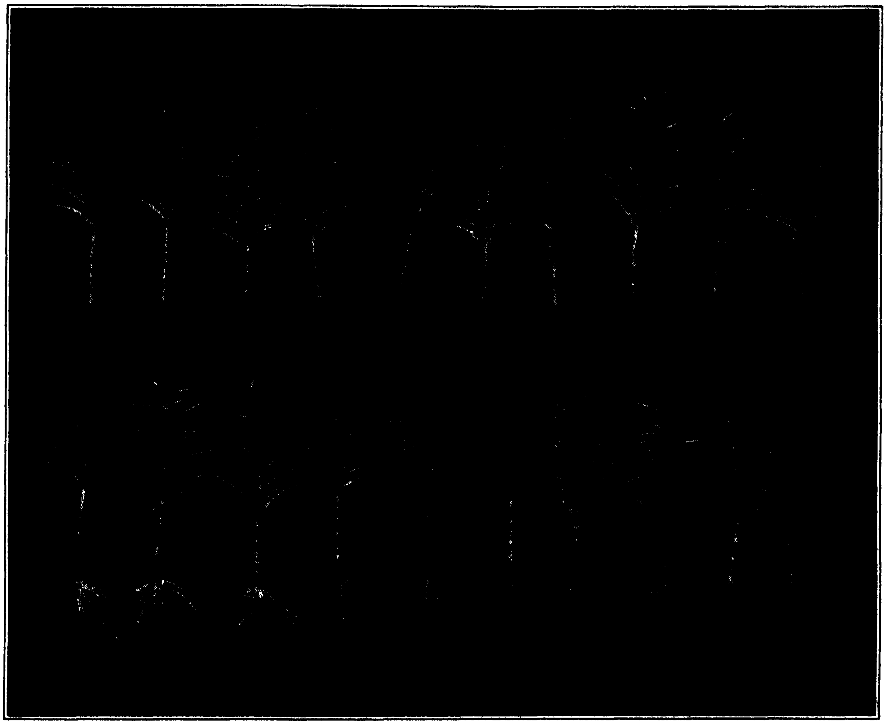


FIGURE 3. Rooting response after two months of *Taxus cuspidata* cuttings placed with basal ends in a solution of naphthaleneacetic acid (4 mg. per 100 cc.) for 24 hours, then planted in peat moss-sand mixture. Top row, controls.

solutions of the growth substances for from one to three weeks indicated that while it was slightly effective for some of the hardwood types without leaves, the difficulty in preventing contamination makes this method less useful than the method whereby relatively high concentrations are used for short periods of a few hours or a few days. *Cornus sanguinea* L. was one of the few species for which several treatments induced rooting, but even in this case it was only the highest concentrations of the series of indoleacetic and naphthaleneacetic acids which were effective, that is, 0.8 mg. per 100 cc. for 7 days and 0.16 mg. for 21 days. Roots emerged

from cuttings transferred to tap water after treatment as well as from those planted in the peat moss-sand mixture.

In a few tests, cuttings of *Chrysanthemum* (var. Golden Menza) were inverted during the period of treatment with solutions of the growth substance to determine whether the inversion method of Went (9) would be of general application to most species of plants without the recommended use of potassium permanganate and sugar solutions. Cuttings were made during January from vegetative shoots approximately 30 inches long taken from plants which had received six hours of additional light each day. Each shoot was made into from 9 to 10 cuttings three inches in length and all leaves were removed before subjecting the cuttings to treatment. Thus each lot of cuttings contained the segments of stem taken from a single long shoot. After being treated in the inverted position for 6 to 24 hours, the cuttings were then placed in the rooting medium in an upright position so that the upper cut surface was level with the surface of the peat moss-sand mixture. At the end of 17 days there was little or no rooting on control lots, whereas on cuttings placed in tap water containing 6 mg. per 100 cc. of naphthaleneacetic acid there was uniform rooting on lots which had been left in the solution for 18 to 24 hours. It appeared to make little difference whether the cuttings were inverted or upright for a period of 24 hours or whether they were inverted for only 6 or 18 of the 24 hours and remained in an upright position in the test solution for the rest of the 24-hour period—all of these treatments giving the same results. Treatment for only 6 hours in the inverted position caused no root growth, but a similar treatment in the upright position produced roots on two-thirds of the cuttings. The inverted treatment for 24 hours inhibited bud growth and for shorter time periods retarded bud growth proportionally. The retardation of bud growth on the upright-treated cuttings was less pronounced.

Tests in which privet cuttings (*Ligustrum ovalifolium* Hassk.) were inverted during treatment with solutions of indoleacetic acid and naphthaleneacetic acid caused large masses of roots to emerge from the upper part of the cuttings, but there was no root growth from the basal end. In addition to this response bud growth was completely inhibited. Control cuttings placed in tap water for a similar period rooted only at the base and shoots up to three inches in length grew from the upper node. The treatment inducing this type of response was indoleacetic acid or naphthaleneacetic acid at the rate of 4 mg. per 100 cc. for five days. At this high concentration and long time period, marked proliferation occurred on the upper, treated ends of the cuttings, which was accompanied by increased callus formation. On cuttings treated in an upright position roots emerged through bark tissue near the base as well as from the basal end.

Cuttings from long canes of *Forsythia* sp. were also inverted in solu-

tions of naphthaleneacetic acid. After 18 days in the rooting medium, roots had emerged from the base of the upper buds in the case of treatments with 6 mg. per 100 cc. for 24 hours, but not in the case of the 2 mg. treatment for the same period. The higher concentration retarded bud growth. Lower concentrations retarded bud growth to a lesser degree. Control cuttings inverted in tap water showed slight retardation of the growth of the upper buds and in this case buds lower down the stem grew. Some of the cuttings treated in the upright position formed roots at the base of the lower buds. Slight retardation of bud growth also occurred on these cuttings. Roots had not appeared in this time from the woody stems of any of the cuttings.

Tests in which the apical cut surface of cuttings was treated with lanolin preparations of the growth substances, separately, and in conjunction with solution treatments applied to the basal ends, prevented the stem portion above the uppermost node from drying and eventually dying as frequently occurs. Furthermore, these preparations induced callus formation on the apical end or a more pronounced callus formation than resulted from treatment with pure lanolin. Similar treatment of the cut ends of lilac shoots up to three-eighths of an inch in diameter gave the same results. In this case the plants were kept in bright light so that the callus was green in color. Lanolin preparations of indoleacetic acid, indolebutyric acid, and naphthaleneacetic acid containing from 0.5 to 2 mg. per gram of lanolin were all effective in causing callus to form on the cut surface exposed to greenhouse atmospheres.

The effectiveness of water solutions of the growth substances in causing root formation in woody or semi-woody plants was further demonstrated by tests in which the solution was introduced into small twigs through a cut surface. In these tests small vials containing a few cc. of the solution were tied to the twig in such a manner that the overhanging piece above the slit dipped into the test solution. The duration of the treatment was from three to ten days. Tap water was added to the vial when the level of the solution was lowered appreciably. Shoots treated in this manner were severed where the slit was made and were then planted in a mixture of peat moss and sand. No attempt was made to determine the optimum conditions for this method of treatment. *Ilex opaca*, *Gardenia jasminoides* Ellis, and *Chrysanthemum* shoots were treated according to this method. The treated cuttings of these species as compared to the controls showed a much more pronounced root growth, earlier rooting, and root growth from a greater area of stem tissue. On the gardenia shoots which were not cut off from the parent plant, roots emerged from the region where the test solution was admitted. Although this method has a limited application, it was noted that solutions of higher concentration could be used than by the method in which cuttings are placed in the test solutions.

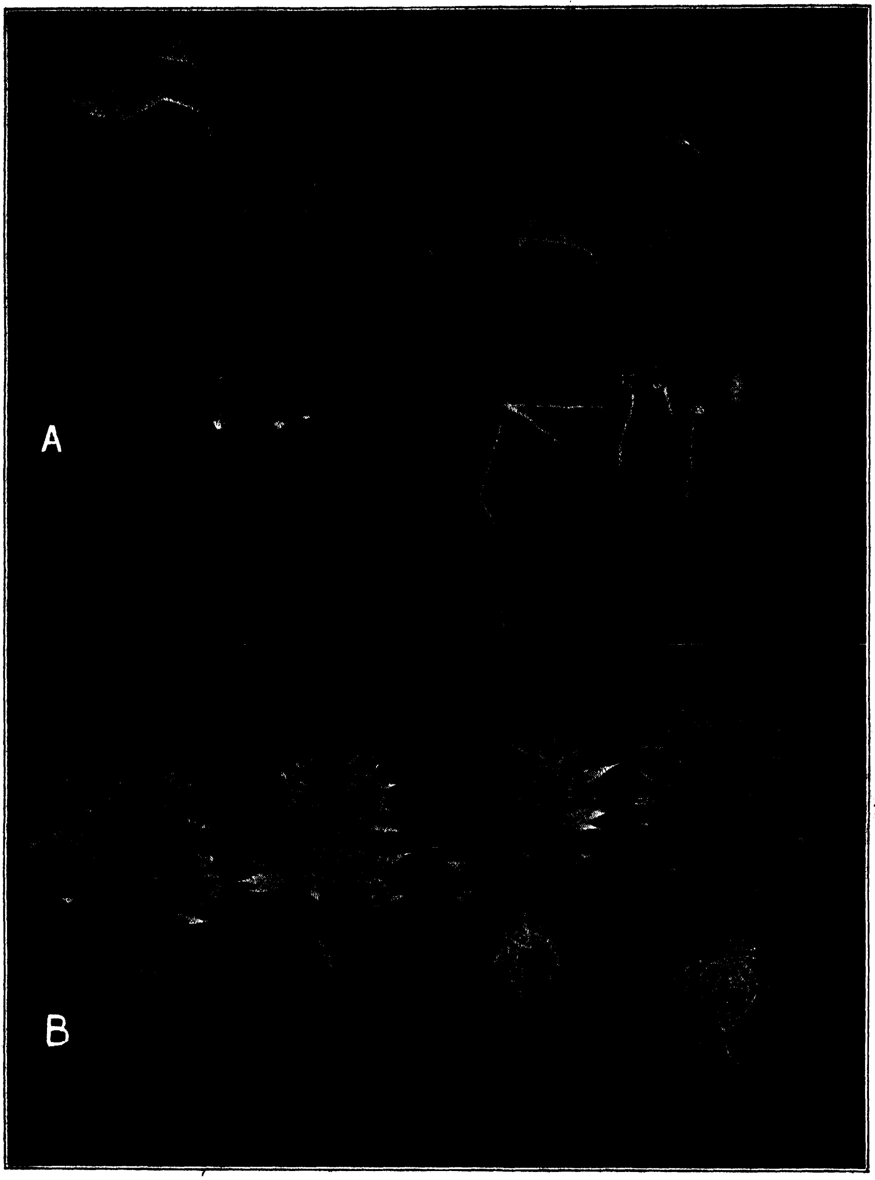


FIGURE 4. Rooting response resulting from treatment of shoots, while still attached to plant, with lanolin preparations of growth substance. A, Grimes Golden apple; left, controls treated at base with pure lanolin; right, treated with indoleacetic acid (100 mg. per gram of lanolin). Shoots remained on plant 13 days after treatment. Photographed 35 days after being in rooting mixture. B, similar treatment of Japanese maple with indolepropionic acid, left to right: control lanolin, 20, 30, and 50 mg. per gram of lanolin. Shoots on plant 22 days after treatment. Photographed 24 days after being in rooting mixture.

LANOLIN PREPARATIONS OF GROWTH SUBSTANCES

Although lanolin preparations of the growth substances are highly effective in causing many types of formative responses on plants, particularly on herbaceous types, they have proved to be relatively ineffective in causing roots to grow on cuttings of many woody species of plants. During the entire growing season of 1935 many hundreds of tests were made with these preparations on commercial fruiting varieties of *Pyrus* and *Prunus*, and on *Crataegus*. With the exception of a few lots of the *Pyrus* (apple) cuttings, lanolin preparations failed to induce rooting on cuttings of this group of plants. A few cuttings of the Grimes Golden (Fig. 4 A), Baldwin, and Jonathan apples were rooted as a result of treatment with a preparation containing from 30 to 100 mg. of indoleacetic acid or indolepropionic acid per gram of lanolin. Two species of the flowering crab (*Pyrus pulcherrima* Aschers & Graebn. and an unknown species, presumably one which is known as *Malus pumila*) also rooted as a result of treatment with these high concentrations of the two indole compounds. When rooted cuttings of the flowering crab were potted, new shoots grew to various lengths up to ten inches.

The synthetic crystalline compounds were mixed with lanolin in amounts ranging from 0.1 to 100 mg. of the growth substance to one gram of lanolin (0.01 to 10 per cent). The vial containing the mixture was immersed in warm water long enough to melt about half of the lanolin and then the preparation was stirred thoroughly with a glass rod. A small amount of this preparation was taken up on a glass rod and applied to the basal portion of shoots which were from two to five inches in length. The length of the treated region was from one-half to three-fourths of an inch. Shoots treated in this manner were cut off just below the treated region at a time ranging from several days to several weeks after the preparation had been applied. Most of the cuttings were then planted in a mixture of half peat moss and half sand by volume. Control cuttings were treated in a similar manner with pure lanolin to which the growth substance had not been added.

Treatment of plants growing outside was made at various times from spring until fall. Plants growing in the greenhouse were treated during the winter as well as at other times.

Japanese maple (*Acer palmatum* Thunb.) was one of the few species of woody plants which responded consistently to treatment with lanolin preparations. Indolepropionic acid was effective in concentrations of from 25 to 100 mg. per gram of lanolin when the duration of treatment on the parent plant was from one to three weeks (Fig. 4 B). Similarly, indoleacetic acid was effective in concentrations of from 10 to 50 mg. These treatments resulted in earlier rooting, the production of more roots per cutting, and the emergence of roots from portions of the stem above the

base of the cutting as well as at the base. Effective treatments increased the average percentage of rooted cuttings from 5, for the control lots, to 46 per cent. The most effective treatments, two of which are shown in Figure 4 B, induced roots to grow on 70 to 100 per cent of the cuttings. Treatment with phenylacetic acid was much less effective than with the two indole compounds named above. Indolebutyric and naphthaleneacetic acids were not available at the time these tests were made. Regardless of whether roots were induced, the high concentrations were noticeably effective in causing an increase in the amount of callus not only on the cut surface but also above this point. These same concentrations generally retarded the elongation of young shoots during the period of treatment on the parent plant. Both of these responses applied to other species as well as to the Japanese maple and are shown for the Grimes Golden apple in Figure 4 A.

DISCUSSION

The effectiveness of certain growth substances in causing roots to be initiated, or in the earlier appearance of the roots in cuttings of several woody species of plants, indicates that this method may be of general application in the vegetative propagation of a number of species which are regarded as difficult to root from cuttings or which require a relatively long period for roots to form. On the other hand, the results also show that the effectiveness of these special growth substances depends to a considerable extent upon the method of applying them to the plant. From a number of standpoints placing the basal ends of cuttings in water solutions of the growth substances is to be considered as a more effective method than the application of lanolin preparations to intact shoots or directly to the cuttings. The solution treatment is much simpler, requires less labor, and appears to be effective in lower concentrations, on more species of plants, and over a wider range of concentrations than lanolin preparations of the same substances.

Although lanolin preparations of growth substances were not applied to holly cuttings taken during December, the results of earlier tests showed little or no favorable influence on the rooting response. In fact, the application of lanolin preparations of growth substances directly to cuttings of woody species has never been so successful as treatment of intact shoots and their subsequent removal from the plant to make into cuttings. The effectiveness of a lanolin preparation when it is applied to the bark of intact shoots, or of cuttings, depends upon penetration of the growth substance to tissue where roots can be initiated. The capacity of lanolin preparations to induce root growth in treated tissue will depend largely upon the permeability of the bark to these substances. Permeability of the bark and also of the underlying tissue to a given growth substance will no doubt vary considerably with the age of the shoot and with different

species. It was shown previously (4) that active lanolin preparations induced root formation only in the upper portions of tobacco stems, but that a profuse growth of roots on the lower part of the stem could be induced by adding a solution of the same substance to the soil. A similar response resulted from the introduction of a water solution of the growth substance through a cut surface made on the lower part of the stem. Furthermore, the effectiveness of a growth substance depends upon the carrier used, olive oil being more efficient than lanolin (4), but olive oil and certain other oils have proved injurious to some plant tissues. When penetration cannot be effected through an impervious bark, mechanical injury, such as slitting in a longitudinal direction or scraping off the outer layer (1), may aid the entrance of the growth substance to the tissue where roots can be initiated. It was observed that callus formation at and near the base of the cutting was greatly increased on those which had been slit in several places before the lanolin preparation was applied. Splitting the end of the cutting was also effective in causing an increase in callus formation on treated stems.

The effective range in concentrations of indoleacetic acid for *Ilex opaca* cuttings was 2 to 40 mg. per 100 cc. and for naphthaleneacetic acid 1 to 20 mg. per 100 cc. when the duration of treatment was from 6 hours to 4 days. No doubt treatment of the holly with lower concentrations for longer time periods would also be effective, since on other species of plants this was found to be true. For example, treatment of *Pachysandra* cuttings for periods of 9 to 12 days with concentrations of naphthaleneacetic acid of 0.2 to 1 mg. per 100 cc. increased root growth. Roots were induced to grow on cuttings of *Cornus sanguinea* as a result of treatment for 7 to 21 days with solutions of indoleacetic acid containing less than 1 mg. per 100 cc. of tap water, whereas control lots of cuttings did not root.

Results with several woody species, including the American holly, indicate that a relatively high concentration for short time periods is likely to be more effective than the use of low concentrations for long time periods. From the practical standpoint, however, the use of low concentrations (approximately 1 mg. per 100 cc.) for several days would be preferable because of the smaller amount of the growth substance required and the greater margin of safety resulting from this type of treatment. Since an 18- to 24-hour treatment with solutions of indoleacetic acid containing 4 to 20 mg. per 100 cc. and of naphthaleneacetic acid containing 2 to 10 mg. per 100 cc. were effective in causing rooting or earlier rooting on cuttings of several woody species, it is believed that these particular treatments will be effective on many other species. With this idea in mind, evergreens such as *Pinus*, *Cedrus*, *Thuja*, *Rhododendron*, etc. have been treated. These and other species will be tested during the entire season of 1936.

According to Went (9) and Cooper (1) the apical ends of cuttings and not the basal ends should be treated with preparations of the growth substances in accordance with their idea that auxins, including indoleacetic acid, can move only downward in stems. Our results with either lanolin or water preparations of the growth substances indicate that a marked increase in root growth was obtained by treating the basal ends of cuttings. These results are in accordance with our previous findings (5) which indicated that synthetic hetero-auxin (indoleacetic acid) and a number of other synthetic growth substances move throughout the plant without exhibiting the unidirectional polar transport claimed by Went (9). Although a growth substance may move longitudinally in either direction from the region of application, it has been observed that the transport to distant points of a sufficient quantity to induce the initiation of roots there, will also cause rooting at the place where the preparation was applied (5). If the concentration of growth substance is excessively high, any roots which may have been initiated in the treated segment of the stem may not emerge from this region or their growth will be noticeably retarded. An example of this type of response is illustrated in Figure 1 B (lot on right) for holly cuttings placed with their basal ends in a high concentration of the growth substance. Privet cuttings inverted during treatment showed a similar response, except that in this case the roots which were retarded or inhibited in growth were located just below the upper cut surface and the roots which grew normally were located from one-half to one-fourth of an inch below the apical cut surface.

It seems unlikely that the treatment of the apical part of cuttings according to the recommendation of Cooper (1) would cause the pronounced root growth on the lower part, as illustrated for holly in Figure 2, without also inducing root growth on the upper treated portion. The fact that Cooper used only lanolin preparations of growth substance, and only one concentration (1:2000), would not be expected to alter the situation. The following statement indicates that Cooper found that root formation in *Acalypha* and *Lantana* cuttings was mainly associated with the treated region at the top (1, p. 793): "Most of the roots on the untreated cuttings appeared at the base of the cutting, while on the treated cuttings roots were scattered all over the internode, appearing mostly at the point of application of the hormone, even when the hormone was applied to a portion of the cutting above ground." The qualitative and quantitative differences resulting from treatment of lemon cuttings having leaves are not clear, since there is no illustration of control lots with which to compare the three treated lots shown.

Treatments which exerted a favorable effect on the rooting response of non-dormant cuttings generally retarded the growth of some or all buds. If the apical ends of the cuttings were treated with either lanolin or water

preparations of the growth substances, bud growth was usually inhibited for a period of at least two to three weeks. This effect was still more pronounced when the bases of the cuttings were placed in the test solution and the apical cut surface was treated with a lanolin preparation of the same or a different growth substance. The use of growth substance preparations to control the growth of buds on cuttings may be of future practical application, particularly in cases where a rapid shoot growth occurs on cuttings which are slow to root. It may mean that cuttings of evergreen plants and of other species made during the months of January, February, and March can be placed at higher temperatures than is the common practice and thus bring about earlier rooting than in the case of the lower temperatures which are used to prevent a rapid shoot growth. Besides retarding bud growth on this type of cutting, the proper treatment with growth substance preparations will no doubt exert a favorable influence on the rooting response. On the other hand, low concentrations might be expected to stimulate bud growth.

Additional data continue to accumulate which show that these growth substances influence the growth of buds. Besides inhibiting the growth of buds, there are several instances in which bud growth has been stimulated by these special substances. In a previous report (4, p. 360), it was shown that axillary buds of the Turkish tobacco formed shoots ranging from 4 to 16 inches in length as a result of treatment of the cut surface with preparations of growth substance after decapitation. One or more shoots of this length grew from as far down as the 11th node and shorter shoots as far down as the 20th node. Application of these same preparations (relatively high concentrations) directly to the bud induced elongation of the bud to a length of one-fourth to three-eighths of an inch and then growth ceased (4, p. 363). Unpublished data, which will appear later in this journal, indicate that some of these substances are highly effective in causing dormant buds of the Jerusalem artichoke to grow. The fact that all of the synthetic growth substances are not equally effective in stimulating the growth of buds points to the possibility that new ones may be found which are still more effective.

The increased callus formation and the favorable preservative effect of lanolin preparations of the growth substances on the cut surfaces above nodes of either cuttings or pruned branches indicates the possible use of this treatment in pruning procedures and possibly in connection with cavity work on trees. Even the use of pure lanolin might aid callus formation in such cases, by preventing undue loss of moisture from the cut surfaces left exposed after pruning or after excavation work on a tree trunk. However, the lanolin preparations containing growth substances would not only retard water loss from the exposed surface, but they would also stimulate callus formation. Some preliminary tests in which graft unions

of *Ilex opaca* were treated with lanolin preparations of indoleacetic, indolebutyric, or naphthaleneacetic acids indicated that this treatment was more effective than the use of paraffin in furnishing conditions favorable for callus formation.

With respect to the practical use of solutions of growth substances for inducing root formation in cuttings, there are a number of facts to be considered. The cost of the growth substances available at the present time may be sufficiently high to be an important factor if treatment is to be made on a large scale basis. In this case it would be preferable to use relatively low concentrations for a period of from one to several days rather than to use relatively high concentrations for a period of 24 hours or less. For example, the highest concentration of the three principal substances (indolebutyric, naphthaleneacetic, and indoleacetic acids) should be from 1 to 4 parts of the growth substance to 100,000 parts of water—that is, the stock solution should contain from 1 to 4 grams per 100 liters (or quarts) of water. Cuttings should be left in these solutions for 1 to 4 days. Some of the stock solution should be diluted 1/2, 1/4 and 1/8 (1:1, 1:3, 1:7) and the cuttings left in these solutions for periods of 1 to 4 days.

Since it has been shown that the effectiveness of these solutions increases with an increase in the concentration up to that which is toxic, there will be a tendency for those who are unfamiliar with the use of these materials to work with concentrations too close to the toxic limits. Likewise, for any given effective concentration, an increase in the duration of the period of treatment will cause a more favorable rooting response up to the time which results in injury to the cuttings. Probably no one treatment will prove highly effective for cuttings of all species or varieties of plants, and it was found that the age and activity of the shoots made into cuttings also determined the effectiveness of the treatment. It would be much safer to make preliminary tests with each species in order to determine the effective range in concentration and the duration of treatment than to treat large numbers of cuttings according to general directions. For this purpose from 5 to 10 cuttings for each treatment will be sufficient. It will be found that certain treatments which are effective on one species will also be effective on a number of other species or genera. The fact that woody cuttings without leaves did not respond to treatment so readily as those with leaves, indicates the importance of leaves in helping to bring about the formation of normal roots in cuttings.

SUMMARY

Treating cuttings or shoots of *Ilex*, *Taxus*, *Pachysandra*, *Hibiscus*, *Acer*, and *Chrysanthemum* with preparations of indoleacetic, indolepropionic, indolebutyric, or naphthaleneacetic acids induced earlier rooting,

increased the number of roots, and roots emerged from a greater area of stem tissue as compared with control cuttings.

Placing the basal ends of cuttings in a tap water solution of the growth substance was particularly effective on *Ilex*, *Taxus*, *Hibiscus*, and *Pachysandra*. This method of treatment is considered as offering much better possibilities for initiating root growth in cuttings than the use of lanolin preparations of the same substances. On a concentration basis, water solutions of the growth substances were from 100 to 500 times more effective than lanolin preparations.

Indoleacetic, indolebutyric, and naphthaleneacetic acids were effective over a concentration range of 10 to 20 times when the period of treatment was from 6 to 96 hours. A 24-hour treatment with a solution containing from 4 to 20 mg. of indoleacetic acid per 100 cc. or 2 to 10 mg. of either indolebutyric or naphthaleneacetic acid per 100 cc. was effective for several species. Solutions containing from 1 to 4 mg. per 100 cc. were equally effective when the duration of the treatment was from 2 to 4 days.

Lanolin preparations of indoleacetic or indolepropionic acid (30 to 100 mg. per gram of lanolin) were consistently effective on *Acer palmatum* but not on many other woody species which were tested during the spring and summer of 1935.

Preparations which were most effective in stimulating root formation also retarded the growth of non-dormant buds.

LITERATURE CITED

1. COOPER, WILLIAM C. Hormones in relation to root formation on stem cuttings. Plant Physiol. **10**: 789–794. 1935.
2. CROCKER, WILLIAM, A. E. HITCHCOCK, and P. W. ZIMMERMAN. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. **7**: 231–248. 1935.
3. HITCHCOCK, A. E. Indole-3-*n*-propionic acid as a growth hormone and the quantitative measurement of plant response. Contrib. Boyce Thompson Inst. **7**: 87–95. 1935.
4. ——— Tobacco as a test plant for comparing the effectiveness of preparations containing growth substances. Contrib. Boyce Thompson Inst. **7**: 349–364. 1935.
5. HITCHCOCK, A. E., and P. W. ZIMMERMAN. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. **7**: 447–476. 1935.
6. ZIMMERMAN, P. W., and A. E. HITCHCOCK. Selection, propagation, and growth of holly. Flor. Exch. **81** (39): 19, 20; (40): 19, 20. 1933. (*Also in* Boyce Thompson Inst. Prof. Pap. **1** (27): 252–260. 1933.)
7. ——— The response of roots to "root-forming" substances. Contrib. Boyce Thompson Inst. **7**: 439–445. 1935.
8. ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.
9. WENT, F. W. A test method for rhizocaline, the rootforming substance. Proc. Akad. Wetensch. Amsterdam, Sec. Sci. **37**: 445–455. 1934.

PROPAGATION OF EPIGAEA REPENS L.
I. CUTTINGS AND SEEDS

FLORENCE L. BARROWS

Coville (14, 15) was the first to grow the trailing arbutus, *Epigaea repens* L., from seed. He also made the important discovery that the plant had a symbiotic root fungus similar to the mycorrhizal fungus of the blueberry. His directions for culture may be found in Bailey's standard cyclopedia (6). Wherry (32), at Coville's suggestion, made a study of soil acidity of the Ericaceae, including *Epigaea*. Councilman (12, 13) studied the roots and clearly showed the coils of an endophytic fungus in the epidermal cells.

COMMERCIALIZATION OF ARBUTUS

As early as 1735 or 1736, John Bartram (7) of Philadelphia is reported to have sent *Epigaea* with other plants to England; but it is only within comparatively recent years that dealers in native plants have commercialized the plant and listed it for sale. The Plant Buyers Index, 1927 edition (22) lists only eight firms as handling trailing arbutus, two firms in Massachusetts, and one each in New Jersey, New Hampshire, New York, Vermont, Ohio, and South Carolina. The third or 1931 edition (23) of this publication lists seven firms, three in Massachusetts, and one each in New Jersey, New Hampshire, Ohio, and North Carolina. The 1934 Supplement (24) does not list *Epigaea* at all.

Commercial prices. A study of the catalogs and price lists of these firms, and some others, with supplementary information from correspondence, reveals twelve firms or individuals handling *Epigaea repens* commercially between the years 1917 and 1935. There is a marked price range over this period and also a good deal of difference in prices between firms. Some firms price plants in small numbers only. Others list large numbers of collected clumps. There is also a marked difference in wholesale and retail prices. A study of the price lists shows the following price range.

For single plants, eight firms, from 1917 to 1935, showed a price range from six cents each for a "special bargain" of 25,000 collected plants, to $1.00 each for two-year and $1.50 each for three-year pot-grown seedlings. By threes, four firms from 1929 to 1935 show a price range of $1.05 to $1.50. By tens, seven firms from 1917 to 1935, show a range from $1.25 for collected plants to $9.00 in pots. By hundreds, ten firms show a range from $7.00 wholesale for collected plants to $70.00 for two-year, and $115.00 for three-year pot-grown seedlings. By thousands, only three firms gave quotations which range from $50.00 for collected plants, wholesale, to $135.00 retail.

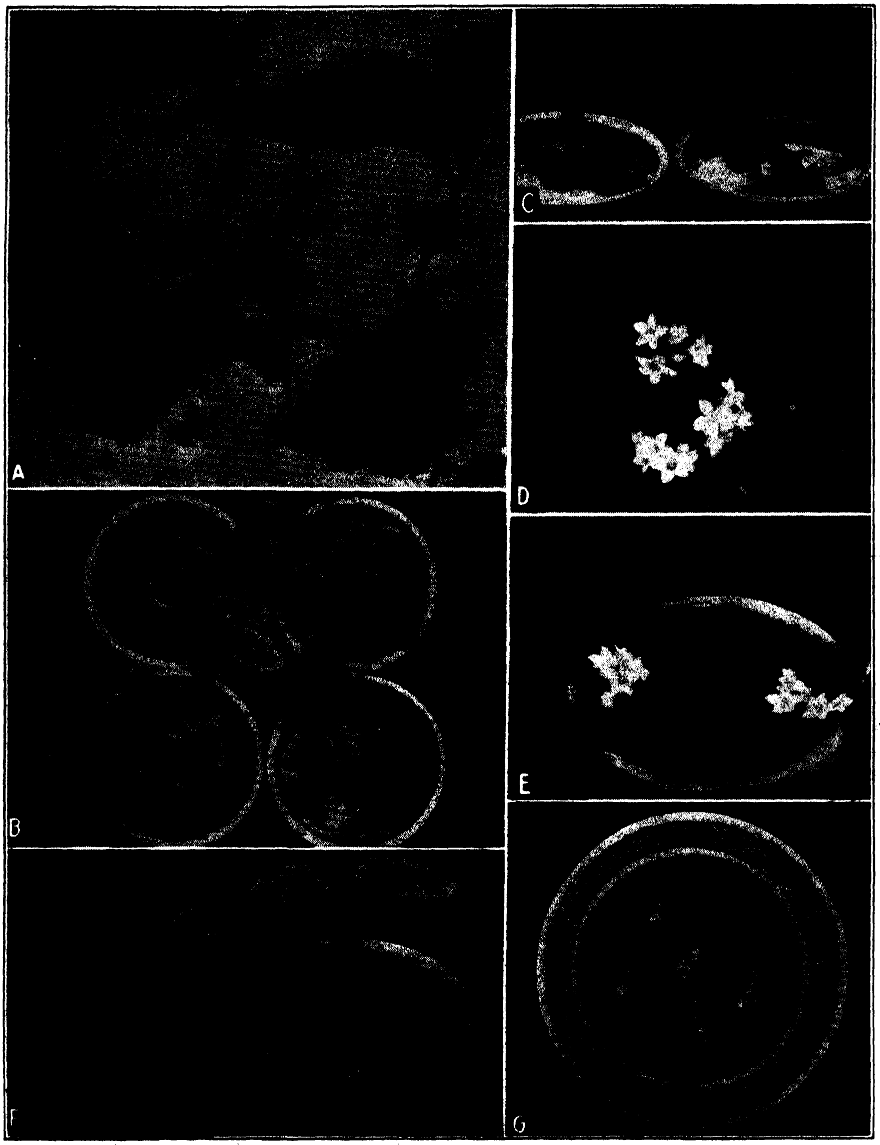


FIGURE 1. A. Arbutus cuttings rooted in peat in cold frame, Sept. 5 to Oct. 29, 1934. ×0.56; B. Plants from cuttings in A grown in greenhouse until June 7, 1935.×0.26; C. Cuttings of Aug. 29, 1933 rooted in Connecticut soil and live sphagnum, and grown in greenhouse until Mar. 23, 1934.×0.28; D and E. Cuttings of Aug. 1933 in flower on Apr. 6, 1935; F. Same plant as E two months later, June 7, 1935 showing new leaf and shoot growth.×0.39; G. Transplanted seedlings in double pot with sphagnum pack.×0.18.

Catalogs usually note that acid soil is needed and one firm gives the warning "very difficult to transplant. Sent only at your risk."

Conservation. From a study of the number of plants offered for sale in the last ten to fifteen years, with consideration of the fact that the great majority of these are "collected" plants, and of the sale of flowers pulled from the woods, those interested in conservation of native flora seem to be somewhat justified in their alarm at the possibility of extinction of this native wild flower. It is one of the plants on the Wild Flower Protection List (May 1, 1932) of the Wild Flower Preservation Society, Inc. This organization publishes a circular "Trailing Arbutus" (28) pointing out the need of protection and giving Coville's methods of germinating seeds. This society is also one of the few sources from which seeds of *Epigaea repens* can be purchased. A package, costing twenty-five cents, was received from them on December 21, 1935. Examination revealed that ten to twelve fruits were enclosed, containing about 1735 seeds. These have been slow to germinate and at the end of two months 5.8 per cent have formed seedlings.

Various garden and horticultural journals publish occasional notes on transplanting (5) or culture (1, 18, 20).

With the increasing interest in the cultivation of *Epigaea repens*, the reported difficulties in transplanting and establishing satisfactory growth, and the receipt of occasional queries as to how these might be overcome, an investigation of the problems connected with propagation was begun. What are the best conditions for rooting cuttings? How can seeds be best germinated? How does the fungus become established in the seedlings?

ROOTING CUTTINGS

The first lot of cuttings tested was collected at a station in northeastern Connecticut on August 29, 1933. The season's growth was completed. After a summer drouth, followed by about a week of heavy rain and damp weather, the tissues were full of moisture and in excellent condition. The material was packed in damp live sphagnum moss and shipped to the Boyce Thompson Institute, where it was placed in a cool room. On September 1, trimmed cuttings were placed in flats in a shaded greenhouse in: live sphagnum, granulated peat moss, Connecticut soil, and granulated peat moss and sand.

Sphagnum. Cuttings placed in live sphagnum on September 1 showed many fine fibrous roots by September 20. The roots were very fine and developed all along the stem or rhizome and clung tenaciously to the sphagnum. On October 4 the rooted cuttings, 18 out of 21, were potted up in three and one-half inch pots in a mixture of equal parts of sand, leaf mold, peat moss, and soil. By November 8 much new hairy tip growth had developed on the shoots. These plants were kept in the greenhouse

during the winter. On February 8, 1934, examination of roots from some of the plants showed the presence of fungus coils. By March 24, five plants had died, some doubtless due to drying out. Thirteen good plants remained in this lot, nine of which had flower buds, formed previous to the time the cuttings were taken, and had made excellent new vegetative growth. On May 11, 1934, these plants were removed to cold frames with slat shade where they were kept for the summer (Fig. 1 C).

Peat moss. The cuttings in peat moss rooted even better than in sphagnum and by September 20, all 22 were transplanted to pots. The roots were numerous and held firmly attached to the peat moss which came up in lumps (Fig. 1 A). The potted plants were treated the same as the lot in sphagnum. Several of these produced flower buds on new shoots.

Connecticut soil. Connecticut soil taken from the station where the cuttings were collected, August 29, 1933, was also favorable to root formation. On October 4, 1933, 19 out of 20 cuttings (2 of which had flower buds) were rooted. These were potted up as described for the lots in sphagnum. In this lot there was also some loss, but on March 26, 1934, 17 good plants remained, five of which showed new flower buds.

TABLE I

EPIGAEA REPENS L. CUTTINGS TAKEN AUGUST 29, 1933

Media	No. of cuttings	No. rooted	% rooted	Placed in media	Date potted
1. Live sphagnum	21	18	85	Sept. 1, 1933	Oct. 4, 1933
2. Peat moss	22	22	100	Sept. 1, 1933	Oct. 4, 1933
3. Conn. soil	20	19	95	Sept. 1, 1933	Oct. 4, 1933
4. Peat moss and sand*	13	12	92	Sept. 7, 1933	Dec. 7, 1933

* This lot was in live sphagnum one week before being transferred to the peat and sand.

Peat moss and sand. A small lot of 13 cuttings which had been in live sphagnum from September 1 to September 7, 1933 was then transferred to a flat with a mixture of equal parts of peat moss and sand. By December 7, 12 were well rooted and covered with masses of fine roots. The other cutting was of old wood, and it was not surprising that this failed to root. It was interesting to note that on two cuttings, roots were formed on petioles which were deeply buried in the medium (Fig. 2 B). On December 18, a fungus was found in and around the roots of one plant sampled. A summary of percentage of rooting of cuttings is given in Table I. The condition of rooted cuttings from sphagnum and Connecticut soil is shown in the photograph (Fig. 1 C) taken March 22, 1934.

From the plants remaining in the cold frames during the summer, there was some further loss, from drying out, especially around the sides of the

frame. Where moisture was greater, there was luxuriant growth of mosses and *Marchantia* on the surface of the soil and even over the *Epigaea* plants. In September 1934, the dead plants were discarded, and the rest weeded to remove the excessive growth of Bryophytes. Most of the living plants had made excellent new leaf growth, dark green in color and of normal size, and had set numerous flower buds. On October 29, the pots of all four sets were sunk in the soil in the cold frame where they remained for the winter. By March 30, 1935, plants in the cold frames were beginning to bloom (Fig. 1 D and E).

Cuttings rooted in the fall and kept in the greenhouse the first winter, where they continued to grow, did much better than those potted up and taken at once to the cold frame. Newly rooted plants, put in the cold frame for the first winter, had a very high percentage of loss, probably because they were not well established and drying out was injurious to them. Cuttings made in the spring of 1934 did not root as well as the cuttings of the previous fall. A small lot of cuttings taken on August 21, 1935, also did poorly. There had been a long period of drouth and the plants were very dry, some of the leaves curled, and the tissues were not well filled with water as two years previous. Only a few of these rooted.

It is interesting to note, however, that a friend was able to root half a dozen cuttings from a bouquet of cut flowers purchased in New York City in the spring of 1935. After the flowers faded, the cuttings were placed in a flat of peat for several months. Two of these cuttings made new growth of rhizomes up to two or three inches in length below the surface of the peat. The new roots were well supplied with the typical fungus coils.

SEED GERMINATION

Pollination

As early as 1796, Michaux (26) while exploring the Allegheny Mountains observed that some plants produced only female flowers which entirely lacked stamens. Other workers who observed flowers from the eastern United States included Gray (16), Ward (31), Bastin (9), and Halsted (17). Wilson (35) published tables showing that the percentage of male and female flowers varied considerably in different localities. Rehder (27) mentioned the finding of a double-flowered form in which the stamens had become petaloid. Burnham (11) found certain plants which bloomed in the fall for several years. Pollen was germinated by Halsted (17) and Andrews (3, 4). Stevens (29) and Andrews (3, 4) studied fertilization and the development of the fruit and seeds. Later Bergman (10) found internal stomata in Stevens' slides of the fruit. Meehan (25) noted that only about half the plants set fruit. Langdon (19) observed four regions where staminate flowers were more abundant than pistillate, and considered that

the scarcity of fruit resulted from the lack of self-fertilization and the infrequency of cross-fertilization.

The flowers of all the plants started from cuttings in August 1933 proved to be pistillate. As the cuttings came from one clump and probably from a single clone, this was not surprising. Unless pollen could be secured from some other source, the plants were incapable of setting seed. These plants in the cold frame flowered earlier than any plants outdoors in the vicinity. Florists in and around New York were unable to supply any cut flowers as a source of pollen. Finally a few flowers shedding pollen were obtained on April 17th from the Arboretum. Pink flowers had pistils longer than the stamens, while white flowers had short pistils. Fourteen plants were brought into the laboratory from the cold frame and pollinated between 5:30 and 6:00 p.m. There was not enough pollen to use on all the open flowers. The pollinated clusters were tagged. The optimum conditions for pollination of *Epigaea* were unknown, but evidently the stigmas had remained receptive for several days. The plants were kept in the laboratory until April 21, when the flowers began to drop and the ovaries to enlarge. They were then returned to the cold frame.

Fruits

By May 3, 1935 seven of eight pollinated plants had set fruits (Fig. 2 A). The numbers of fruits set per plant varied from none to eight and gave a total of 26 fruits set. On May 8, two of the developing fruits were preserved for later study. The fruit on the plants was examined at various intervals and seemed to be developing normally. New leaves were expanding rapidly (Fig. 1 F). Some foliage was injured by handling during photography, but most of the leaves were larger and more healthy in appearance than those of the previous season's growth. The first fruits opened on June 10, and three were harvested on that day (Figs. 2 A and 3 B). One was wide open, one partly open with the style still attached, and the other was abnormal in showing four carpels instead of the typical number, which is five. During the next week all but ten fruits on three plants were harvested. On June 21, the writer left to collect seed in Connecticut. At this time there were still immature fruits on the cold frame plants. Several of these never did ripen, but by July 25 had turned brown and were obviously not good. Some of them were on two plants which had been handled considerably while being photographed and were exposed temporarily to intense light and heat.

Seeds

As the fruits were harvested, each one was placed in a clean sterile petri plate and examined microscopically, and the seeds counted as soon as opportunity permitted. A Spencer binocular with 12.5 oculars and $\times$1.0

or higher objective was found very satisfactory for dissecting the seeds out of the fruits, and a reading glass was useful for counting the seeds. An illustration in color by J. M. Shull and description of *Epigaea repens* fruit appeared in the National Geographic Magazine, May 1915 (15, p. 506). When mature, the five carpels of the fruit first crack apart, and then roll back exposing the brown seeds imbedded in depressions in the white sticky

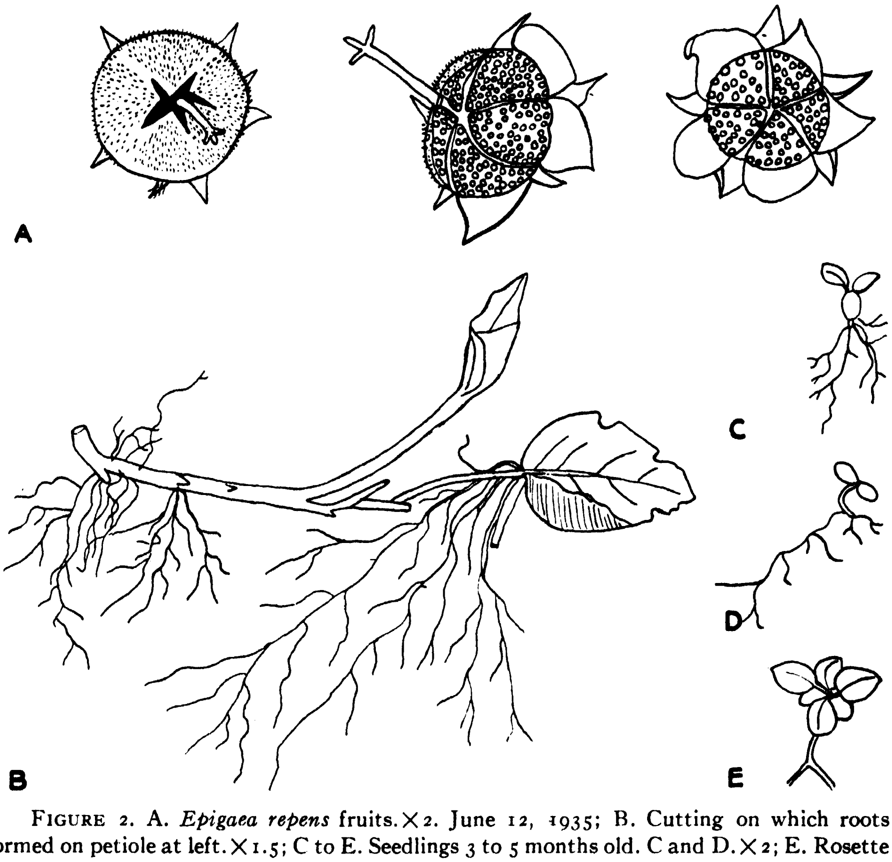


FIGURE 2. A. *Epigaea repens* fruits. ×2. June 12, 1935; B. Cutting on which roots formed on petiole at left. ×1.5; C to E. Seedlings 3 to 5 months old. C and D. ×2; E. Rosette of 6 leaves. ×1.

pulp. The seeds are very shiny and have a hard brown coat which varies in color in different fruits. Sometimes seeds will snap off and fly considerable distances when touched with a needle. At other times it is a difficult task to get them freed from the sticky pulp in which they are imbedded. Seeds also stick to the glandular hairs on the outer surface of the carpels. There were fruits where all the seeds were plump, while in others there were a few aborted seeds. In other cases one or more carpels were filled with aborted seeds, the numbers running up to 200 or 250. In some small

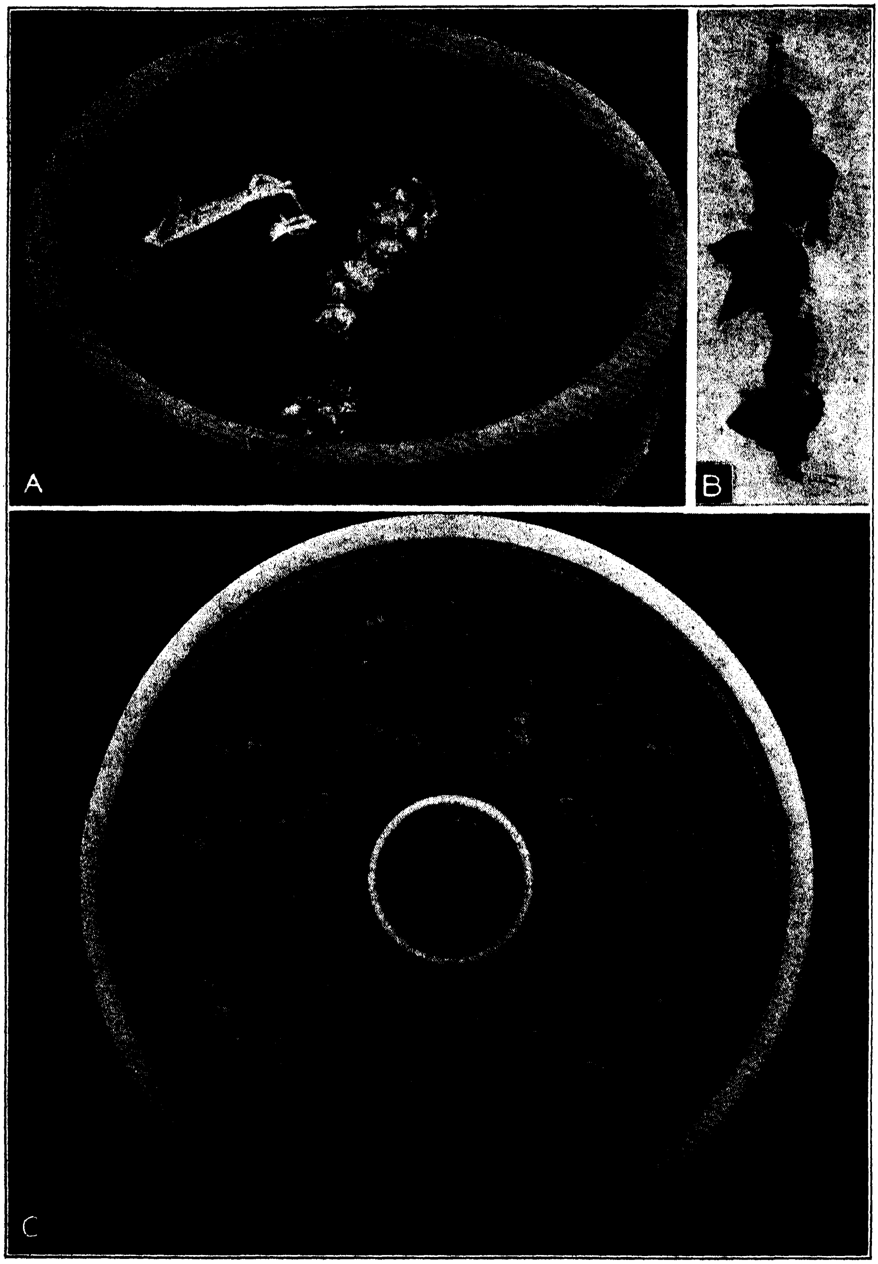


FIGURE 3. A. Young fruits on May 3, 1935 from flowers pollinated April 17.×0.86; B. First fruits ripe June 10, 1935.×1.5; C. Transplanted seedlings at 9 months. Kept moist in double pot with water in center.×0.33.

fruits which failed to mature and open, there was nothing but aborted seeds.

Seed count. Seeds were counted by the method used by Miss Clyde Chandler of the New York Botanical Garden for counting *Petunia* seeds. After the seeds were dissected out into a petri plate, a clean scalpel was used to push them into groups of 10, which were later pushed together into groups of 100 around the edge of the plate. The reading glass mentioned above proved an aid in counting, as the seeds are quite small.

Of the 14 ripe fruits resulting from hand pollinations, the total yield of seeds was 5641 or an average of 403 seeds per fruit. The range was from 177 to 616 seeds. Four plants produced more than one mature fruit. Three plants yielded two each, the total number of seeds per plant being 875, 927, and 959 seeds. One plant produced three fruits, yielding a total of 1339 seeds. All these plants were from single branch cuttings made in August 1933 and were in three and one-half inch pots at the time of fruiting in June 1935 (Fig. 3 A).

Fruits from Wild Plants

During the last week of June 1935, *Epigaea repens* fruits were collected in Union, Connecticut. Most of the fruits were secured from plants growing in an old pasture on a slight slope facing north. They were partly shaded by white pine, mixed hardwoods, and such shrubs as *Kalmia latifolia* and *Vaccinium*. The dates of collection were June 26 and June 29, most of the fruits being secured on the later date. Some fruits had already matured and only empty capsules were left on the plants. On other plants the fruits were still green and immature. Many small black ants were working on the pulp of ripe fruits. Chipmunks were also at work on the fruits. These and other animals doubtless play some part in the distribution of the seeds. The fungus which is present in the fruits and on the seed coats, may also be scattered by this means. Over 160 fruits were collected in glass vials. In the short time available it was impossible to ascertain how many fruits were produced per plant, as the plants were often in mats and tangled masses. However, where several fruits were present in a cluster, these were kept together to determine branch yield. In some cases two or three empty capsules on a branch were accompanied by one or two immature fruits. In some instances, all but the contents of one or two of the five carpels had disappeared. The seed counts of 155 collected fruits were recorded as being reasonably complete, and presenting a fair picture of the average yield per fruit. The 155 fruits contained 37,406 seeds, an average of 241 per fruit. The range was from 29 in a small but mature fruit to one containing 415 seeds. This range is more extreme and not as high as for the hand-pollinated fruits mentioned above. Of course the number of hand-pollinated fruits was only 14 as compared to the 155 here

considered. The accompanying graph (Fig. 4) shows the distribution of seed numbers in these collected fruits.

Branch yield. The greater number of these 155 fruits were single ovaries; but it is interesting to note that there were 7 clusters of 2, 6 of 3 fruits, 2 of 4 fruits, 2 of 5 fruits, and one each of 6 and 8 fruits. In these clusters, the average yield per branch was respectively 394, 756, 1028, 1306, 1251, and 2065 seeds. So it is safe to conclude that a single plant can produce up to 1000 or 2000 seeds. In the seed counts recorded only plump seeds were included.

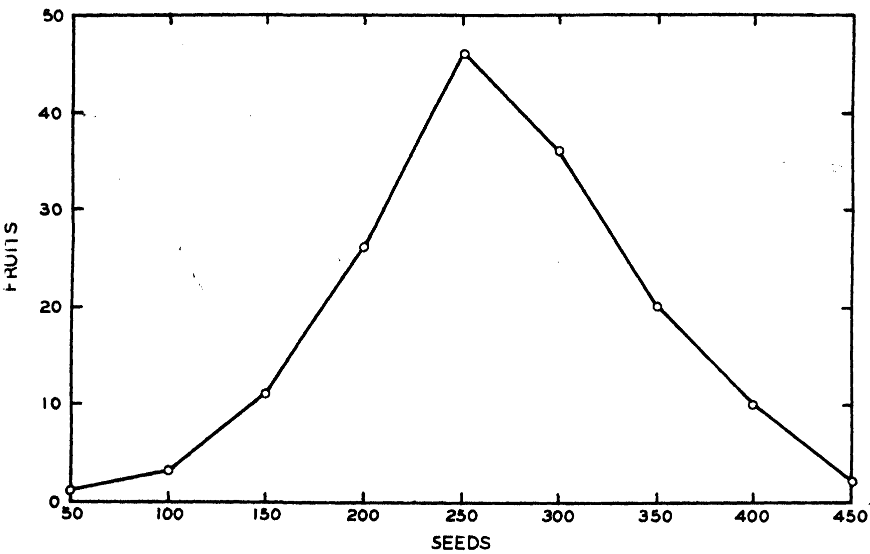


FIGURE 4. Seed yield in 155 *Epigaea repens* fruits.

These yields are interesting in comparison with a statement made by Mr. Robert S. Lemmon on Dec. 18, 1935, in his lecture before the Horticultural Society of New York on "Some Outstanding Native American Plants." In connection with a lantern slide of fruit of the trailing arbutus, he reported 100 to 150 seeds from fruits on pot-grown plants. Ricker (28) reports 20 to 100 seeds as typical of wild plants. Coville (15) says a fruit bears usually 300 to 500 seeds.

GERMINATION

The first *Epigaea* seeds obtained during this work were found December 26, 1934, on a plant collected November 25, 1934, at Union, Connecticut. The plant grew on a bank by the road-side under a rock, and at the time of collection the fine roots were frozen to the stone so that it was impossible to get all of them. The plant was shipped to the Boyce Thompson In-

stitute and potted up November 30, and placed in the greenhouse. By December 26, the flower buds were enlarging and beginning to show color. In an old flower cluster, one old fruit, very dry and partly gone as though it had been eaten, was found to still contain about 40 to 50 seeds. These had already been exposed to freezing temperatures and to two or three snow storms before the plant was collected. In order to watch for possible germination these seeds were sown on a piece of moist filter paper laid on the top of a two and one-half inch pot filled with moist peat moss and placed in a moist chamber at room temperature on the laboratory table. On January 16, 1935, the first germination was noted. Several seedlings

TABLE II

EPIGAEA REPENS L. SEED GERMINATION 1935

No. of fruit	Collected	Planted	Media	No. of seeds	No. of seedlings	Per cent germination	Counted
1	June 10	June 18	Soil mixture double pot	360	170	47.2	Sept. 4
2	June 10	June 20	"	343	178	51.8	Sept. 4
3	June 10	June 20	"	380	128	33.6	Sept. 4
4	June 11	July 5	Filter paper over peat moss	177	84	47.4	Aug. 31
5	June 12	July 5	"	281	1	0.3	Aug. 31
6	June 12	July 5	"	565	270	47.7	Aug. 31
7	June 13	July 5	"	339	286	87.3	Aug. 31
					Av.	45.0	
8	June 14	Sept. 11	Soil mixture double pot	435	31	7.1	Oct. 28
9	June 13	Sept. 11	"	515	145	28.1	Oct. 28
U-1	June 29	Sept. 11	"	356	212	59.5	Oct. 28
U-2	June 29	Sept. 11	"	357	158	44.1	Oct. 28
					Av.	36.7	

were transferred to a drop of water on a slide by means of a camel's hair brush, and studied microscopically. Accurate counts were not made, but the per cent of germination must have been high as few seeds failed to germinate.

From the seeds collected in 1935 and previously mentioned under *Fruits* and *Seeds*, germination tests were made as indicated in Table II. The range of germination was from 0.3 to 87.3 per cent, with an average of 40.4 per cent. This included a test of 4118 seeds from 11 fruits. As previously indicated the seeds of each fruit were counted separately in a petri plate and stored either at room temperature or in the refrigerator until sown. Seven lots were planted in double pots, containing a center two and a half inch pot corked to hold water (30), and surrounded by the soil formula previously found satisfactory for rooted cuttings, i.e., equal parts

of peat moss, sand, leaf mold, and No. 2 sod. The seeds were scattered over the surface of the soil and no attempt was made to cover them. The whole pot was enclosed in a moist chamber, or a glass crystallizing dish was inverted over the top. Four lots were sown on moist filter paper over moist peat moss, as in the preliminary test made in December 1934. These peat moss cultures gave the greatest range of per cent germination, i.e., 0.3 to 87.3, both the highest and the lowest of any of the tests. The lowest germination was on a paper which became covered with a gray fungus, which eventually entirely destroyed the paper, and was apparently unfavorable to germination. That some of the seeds in this fruit were viable is indicated by the fact that at least three germinated. In some cases a higher count might have been obtained by waiting until a later date for count of the seedlings. Germination began to show in lots 4, 6, and 7 on August 13; in lots 8, 9, U-1, and U-2 on October 15. The most rapid germination was observed on paper over peat moss. Possibly this was because germination was easier to see here on a light background than on the soil mixture which was so nearly the color of the seeds. The period for germination varied from about 21 to 66 days. The general impression seems to be that seeds should be planted as soon as harvested (1, 2, 6, 15). However, germination slightly better than the average occurred in two lots of wild seeds held until Sept. 11. Three lots started on December 24 and counted two months later, ranged from 0.6 to 27 and averaged 13.5 per cent. Seeds of 80 fruits started January 9, showed germination in only 5 lots by the end of 7 1/2 weeks and these averaged only 2 per cent germination. Aged seeds are being held and tested at various intervals to determine duration of viability. So far the older seeds are slower and much more irregular in germination, as well as showing decreased germination.

DEVELOPMENT OF THE SEEDLINGS

After the two green cotyledons appear, the root soon begins to branch and form secondaries and the beginning of a delicate fibrous root system. On lots 1 and 2, started June 18 and 20, 1935 respectively, the third leaf, i.e., the first foliage leaf, showed on September 17, 25 days after germination was first visible (Fig. 2 C to E). On the December 26, 1934 preliminary tests, a few seedlings were transplanted to a pot sunk in a larger pot packed with damp sphagnum (Fig. 1 G). The others were transplanted later to a 10-inch pot with central water cup as shown in Figure 3 C. By this time the paper was much softened, and many roots had already penetrated the peat. These plants would probably have grown more rapidly if they had been transplanted earlier. On August 9, 1935, peat from this seed pot, which was known to contain some broken roots from the transplanted seedlings, and which was also known to contain the mycorrhizal fungus, was sprinkled over the surface of pot 2 mentioned

above. This pot gave slightly higher percentage germination than the other two planted at about the same time, and the seedlings made more rapid growth and were somewhat larger and better developed than the other sets.

Transplanting seedlings. As the seedlings germinated on paper over peat had little nutrient material available and were in danger of being over-run by fungi in the moist chamber, it was necessary to transplant them earlier than when they were germinated on soil. They are small and hard to handle at this stage, as they have to be moved under a hand lens by means of a camel's hair brush or a forceps, and there is danger of bruising or injuring them. They must also be prevented from drying out. Seedlings germinated in the soil can be left much longer, until the third or even the fifth or sixth leaf appears before they become too crowded (Fig. 2 C to E). After transplanting seedlings it is well to keep them covered with glass until growth is renewed, and then to remove the protection gradually to prevent drying out. The moisture conditions most favorable for the growth of the arbutus seedlings also encourage the growth of algae, fern prothallia, and an especially luxuriant growth of mosses, so these may have to be weeded out if they become too rampant.

ENVIRONMENTAL FACTORS

Soil acidity. As previously mentioned, Wherry (32) in his study of soil acidities made a few tests for *Epigaea repens*. In the Willoughby Lake region, Vermont, he states (32, p. 40) "Upland peat with subacid reaction on the slopes of the hummocks of drift supports *Epigaea repens*. . . ." His Table III (32, p. 44) indicates this plant capable of growing at the "specific

TABLE III
SOIL pH OF EPIGAEA REPENS L. STATIONS 1934

March 30	April 28	May 27–29	July 29–31	Sept. 3	Oct. 1	Nov. 25
7.67	6.97	7.09	7.11	6.58	6.85	5.40
7.27		7.04	6.95	6.50		
		7.04	6.94			
		6.96	6.92			
		6.94	6.79			
		6.67	6.71			

acidities of 300—mediacid" and "30—sub-acid," with the optimum at 100, but able to grow temporarily at 10 (32, p. 47) in Gillett's nursery at Southwick, Massachusetts. In 1927 (34, p. 197) the mediacid is given as pH 4.1 to 5.0 and the sub-acid as 5.1 to 6.0.

During the season of 1934, while soil pH values were being determined for *Lycopodium* (8, p. 288 Table VII) tests were also made as opportunity offered on *Epigaea* which was often found growing in or near the *Lyco-*

podium stations. During the period from March 30 to November 25, 1934, the range was from pH 7.67 to 5.40, showing a decided trend toward greater acidity as the season advanced. These figures are based on 19 determinations as indicated in Table III and were made by the Youden hydrogen ion concentration apparatus (36). However, rooted cuttings and seedlings grown in the soil formula of equal parts of peat moss, sand, leaf mold and No. 2 sod, with pH 4.65, thrived and did well. Peat moss in a flat where arbutus cuttings were held from April 4 to September 5, 1934 gave pH 6.52. Fresh unused peat moss in which cuttings were started September 1934 had a pH value of 6.53. This same peat moss on October 29, when the cuttings were well rooted and potted, gave pH 6.46.

Two seedlings which had been germinated in 1932 in the Institute seed laboratory according to Coville's directions in Bailey's cyclopedia (6) were potted in soil of pH 5.83 (calomel electrode). These plants were found on October 30, 1934 to have soil pH values of 5.92 and 5.28 (quinhydrone method). At that time they had little or no fungus in the roots. Peat moss and sand in which cuttings had been rooted, and still containing broken roots with fungus, were added to the surface of one pot, the other being reserved for a control. The results of the fungus inoculation test will be discussed in a later paper on the endophytic fungus.

The rather wide range of pH values under which *Epigaea repens* grows and thrives, indicates that soil acidity, while doubtless an important factor, is not the only necessary condition.

One of the most luxuriant artificial stands of *Epigaea repens* seen by the writer, was located on the Anton G. Hodenpyl estate at Locust Valley, Long Island, where it was well established under white pines (*Pinus strobus* L.) on a ridge. The owner told how the acidity had been increased by mulching with peat and pine needles. This was the estate where the soil acidity was mapped by Wherry (33) in June 1922. The arbutus was growing in the region at the left of his map, where the specific acidity was 300 or about pH 4.5.

Moisture. Moisture seems to be especially important for both seedlings and cuttings. Constant moisture seems to be more essential than an acid soil. With the delicate fibrous character of the roots, this is easily understood. A mulch of peat or pine needles, or similar means of retaining soil moisture, is helpful. Lemmon (20, 21) who grows his seedlings in peat pots, sunk in peat in cold frames, gives special directions for watering the first season after transplanting. Aiken (2) in his cultural directions also emphasizes the need of moisture. The writer's experience confirms this. In nature thrifty plants are found in moist but well drained places, such as along the sides of old logging trails, on hummocks in low lands, or on sloping pastures.

Shade. Wild arbutus seldom grows in the open, in full sunlight. Some

shade seems to be necessary. Plants are usually so located as to receive shade at least part of the day. In very dense shade the leaves may be luxuriant, but such plants seldom produce flowers as do those which get partial shade. Both seedlings and cuttings were grown successfully in our orchid house with shaded glass. Well established rooted cuttings did well in the cold frame with slat shade and a pine needle mulch. Aiken (2) seems to have solved the problem commercially by use of a brush shed as indicated in his Plate XIII of "Trailing Arbutus growing in the Brush House at Putney."

SUMMARY

1. Trailing arbutus (*Epigaea repens* L.) may be propagated successfully by cuttings provided certain precautions are taken. A sufficient supply of moisture must always be present, and protection from too intense sunlight. Probably the presence of a mycorrhizal fungus in the soil is also essential. All vigorously growing plants which were examined were found to have well developed coils of fungus in the roots. This is true of collected plants as well as those produced by either cuttings or seeds. Stunted plants lacked fungus in the roots or had very little present. The endophytic fungus relations will be discussed more in detail in a later paper.

2. Cuttings root readily along the rhizome when placed in live sphagnum, native soil, peat moss, or peat moss and sand. Cuttings made in late August included the current and previous season's growth. August or September cuttings gave a higher percentage of rooting than spring cuttings. Rooted cuttings kept in the greenhouse the first winter continued growth and did better than similar cuttings in the cold frames.

3. There was a big difference in the percentage of germination of seeds from different fruits. The range was from 0.3 to 87.3 per cent for individual fruits. The germination was higher in seeds planted soon after the fruits matured. Seeds from seven fruits planted by July 5 averaged 45 per cent. Four lots started September 11 averaged 36.7 per cent. Three lots, after 6 months' storage, started December 24, averaged 13.5 per cent germination two months later. Eighty lots started January 9, showed germination in only 5 at the end of 7 1/2 months, and these averaged only 2 per cent germination. Aged seeds are still being tested to determine the period of viability. Seeds can be germinated either on moist filter paper over peat or in a soil mixture. For the amateur the soil method is preferable as transplanting can be delayed until the plants are larger and easier to handle. Moist chambers or double pots help to maintain the moisture so essential to development.

4. A total yield of 37,406 seeds was produced by 155 fruits collected from wild plants, giving an average of 241 seeds per fruit. Branches bearing clusters of fruits were found to yield as high as 2065 seeds. Hand

pollinations resulted in fruits yielding from 177 to 616 seeds, averaging 403 seeds per fruit, and as high as 1339 seeds per plant.

5. The soil acidity of stations of *Epigaea repens* in Union, Connecticut from March 30 to November 25, 1934, was found to range from pH values of 7.67 to 5.40 as based on 19 determinations. Cuttings and seedlings grew well in soil mixture of pH value 4.65. Thrifty growth of *Epigaea* may be maintained over a wide range of soil acidity.

LITERATURE CITED

1. AIKEN, GEORGE D. Growing the trailing arbutus. Home Acres **20** (5): 6. 1932.
2. ——— Pioneering with wildflowers. 2nd printing 131 pp. Publ. by the author, Putney, Vt. 1935.
3. ANDREWS, F. M. Die Anatomie von *Epigaea repens*, L. Beih. Bot. Centralbl. Abt. 1. **19**: 314–320. 1906.
4. ——— The embryo-sac and pollen of *Epigaea repens*. Beih. Bot. Centralbl. Abt. 1. **44**: 264–266. 1927.
5. ANDREWS, MILDRED. Trailing arbutus—plant of the forest. Gard. Chron. America **34**: 194–195. 1930.
6. BAILEY, L. H. The standard cyclopedia of horticulture. 3rd ed. v. 2. p. 1120–1121. Macmillan Co., New York. 1919.
7. BARNHART, JOHN HENDLEY. Significance of John Bartram's work to botanical and horticultural knowledge. Bartonia. Suppl. to No. 12: 24–34. 1931.
8. BARROWS, FLORENCE L. Propagation of *Lycopodium*. I. Spores, cuttings, and bulbils. Contrib. Boyce Thompson Inst. **7**: 267–294. 1935.
9. BASTIN, EDSON S. Structure of *Epigaea repens*. Amer. Jour. Pharm. **67**: 231–236. 1895.
10. BERGMAN, H. F. Internal stomata in ericaceous and other unrelated fruits. Bull. Torrey Bot. Club **47**: 213–221. 1920.
11. BURNHAM, S. H. Notes on *Epigea repens* L. Torreya **4**: 25. 1904.
12. COUNCILMAN, W. T. The root system of *Epigaea repens* and its relation to the fungi of the humus. Proc. Nat. Acad. Sci. **9**: 279–285. 1923.
13. ——— The relation between the roots of plants and fungi. Proc. Soc. Exp. Biol. & Med. **21**: 361–363. 1924.
14. COVILLE, FREDERICK V. The use of acid soil for raising seedlings of the mayflower, *Epigaea repens*. Science **33**: 711–712. 1911.
15. ——— The cultivation of the mayflower. Nation. Geog. Mag. **27**: 518–519. 1915.
16. GRAY, ASA. Heteromorphism in *Epigaea*. Amer. Jour. Sci. III. **12**: 74–76. 1876. (*Same as* Amer. Naturalist **10**: 490–492. 1876.)
17. HALSTED, BRYON D. Notes upon *Epigaea repens*. Bull. Torrey Bot. Club **18**: 249–250. 1891.
18. KNOWLTON, FRED G. The trailing arbutus—(*Epigaea repens*). Flower Grower **18**: 190. 1931.
19. LANGDON, FANNY E. A study of *Epigaea repens*. Asa Gray Bull. **4**: 1–3. 1894.
20. LEMMON, ROBERT S. The trailing arbutus in home gardens. Horticulture **13**: 101–102. 1935.
21. ——— Peat moss and earthworms. Horticulture **13**: 482. 1935.
22. MANNING, J. WOODWARD. The plant buyers index. 127 pp. looseleafed. Gerald Guy Manning Co., North Cambridge, Mass. 1927.
23. ——— 3rd ed. J. W. & E. C. Manning, Reading, Mass. 1931.
24. ——— 1934 Supplement. J. W. & E. C. Manning, Duxbury, Mass. 1934.

25. MEEHAN, THOMAS. Variations in *Epigaea repens*. Proc. Acad. Nat. Sci. Philadelphia. **19**: 153–156. 1868.
26. MICHAUX, ANDRÉ. Portions of the journal of André Michaux, botanist, written during his travels in the United States and Canada, 1785–1796. Proc. Amer. Phil. Soc. **26**: 1–145. 1889.
27. REHDER, ALFRED. New species, varieties and combinations from the herbarium and the collections of the Arnold Arboretum. Jour. Arnold Arbor. **7**: 239–244. 1926.
28. RICKER, P. L. Trailing arbutus. Wild Flow. Preserv. Soc. Circ. 19. 3 pp.
29. STEVENS, NEIL E. Dioecism in the trailing arbutus, with notes on the morphology of the seed. Bull. Torrey Bot. Club. **38**: 531–543. 1911.
30. TEUSCHER, HENRY. A simple aid in propagation. Jour. New York Bot. Gard. **36**: 120–122. 1935.
31. WARD, LESTER F. Sexual differentiation in *Epigaea repens*. Amer. Naturalist **14**: 198–200. 1880.
32. WHERRY, EDGAR T. Soil tests of Ericaceae and other reaction-sensitive families in northern Vermont and New Hampshire. Rhodora **22**: 33–49. 1920.
33. ——— A soil acidity map of a Long Island wild garden. Ecology **4**: 395–401. 1923.
34. ——— Divergent soil reaction preferences of related plants. Ecology **8**: 197–206. 1927.
35. WILSON, W. P. Observations on *Epigaea repens*, L. Contrib. Bot. Lab. Univ. Pennsylvania **1**: 56–63. 1893.
36. YOUDEN, W. J., and I. D. DOBROSCKY. A capillary glass electrode. Contrib. Boyce Thompson Inst. **3**: 347–362. 1931.

GRAVITY-POSITION OF TOMATO STEMS AND THEIR PRODUCTION OF THE EMANATION CAUSING LEAF EPINASTY[1]

F. E. Denny

In previous experiments (2, 3), it was found that the leaf epinasty caused by low concentrations of ethylene, and by contact with the air surrounding apple fruits (Botjes, 1) because of the ethylene produced by the apples (Gane, 5), was produced not merely by the volatile products from fruit tissue but by those from various plant organs, including leaves, stems, roots, petals, pistils, etc.

Subsequent experiments with young tomato stems from which the leaves had been removed have shown that the rate of production of the emanation depended upon the position of the stem toward gravity, the horizontal position being more effective than the vertical.

METHODS

The apparatus used for accumulating the gaseous products from the tomato stems, and applying them to potato plants for observations as to epinastic response, is shown in Figure 1. The tomato plants (*Lycopersicon esculentum* Mill.) used for obtaining a supply of stems were such as had been spaced at two-inch distances in transplanting them into flats. When selected for the tests they were 8 to 12 inches high and the stems were rather slim. The young stems, after removal of the leaves, were first sorted on a balance into paired lots having the same number, weight, and length of stems. They were then tied with cord to the glass rod in the center of the large tubes (Fig. 1 A), one sample of each pair being placed in the horizontal position, and the other in the vertical position as shown in Figure 1 A. The tubes were covered with black cloth and were allowed to stand in the two positions for 24 to 48 hours, after which the air surrounding the stems was displaced into the 750 cc. desiccators or liter pyrex Florence flasks (Fig. 1 B and C) which contained the tips of young potato plants that were to be used as test plants for the presence of emanations causing leaf epinasty. The potatoes (*Solanum tuberosum* L.) grew in flats until the sprouts were about 3 to 5 inches high; these were cut off at the surface of the soil, were supported by bits of cotton in vials of water, and were placed in the vessels as shown in Figure 1 B and C. The desiccators or flasks containing the potato tips were evacuated,[2] and by connecting the evacuated vessel to the outlet of the long tube containing

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 108.

[2] The Florence flasks were covered during evacuation to avoid danger of injury by breakage.

the tomato stems any desired aliquot of the air surrounding them could be transferred by admitting a measured amount of water to the inlet tube, care being taken to do this slowly, and to see that atmospheric pressure was obtained at the end of the transfer as shown by the external water level. After the desired amount of air, corresponding to a definite weight of the stem tissue, had been admitted to the desiccator or flask, the vessel

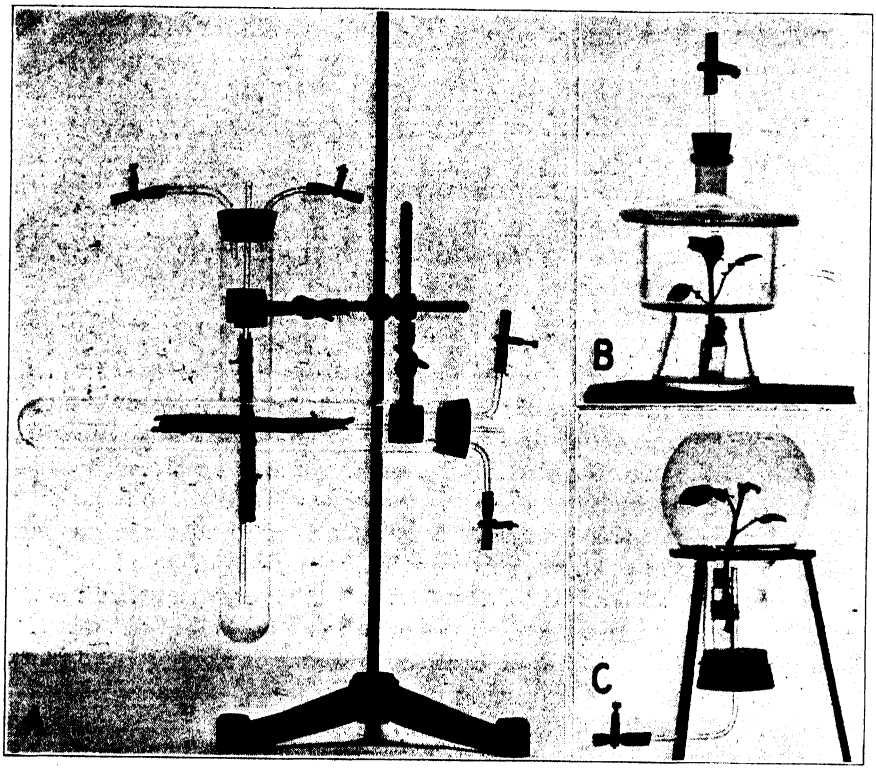


FIGURE 1. Apparatus for accumulating volatile products from tomato stems and applying them to potato test plants. A. Large test tubes (approx. 700 cc.) containing tomato stems sorted into paired lots of same number, length, and weight, one lot placed in horizontal, other lot in vertical position. B and C. Vessels containing potato test plants for response to the air surrounding the tomato stems in A.

was carried to a greenhouse, or to the outdoors, and the vacuum was released.

The desiccators and flasks were placed on the laboratory table in positions at random as regards the various lots and were covered with black cloth. The first signs of epinastic response usually occurred within about six hours and the response, if it was to be obtained at all, was complete within about 30 hours.

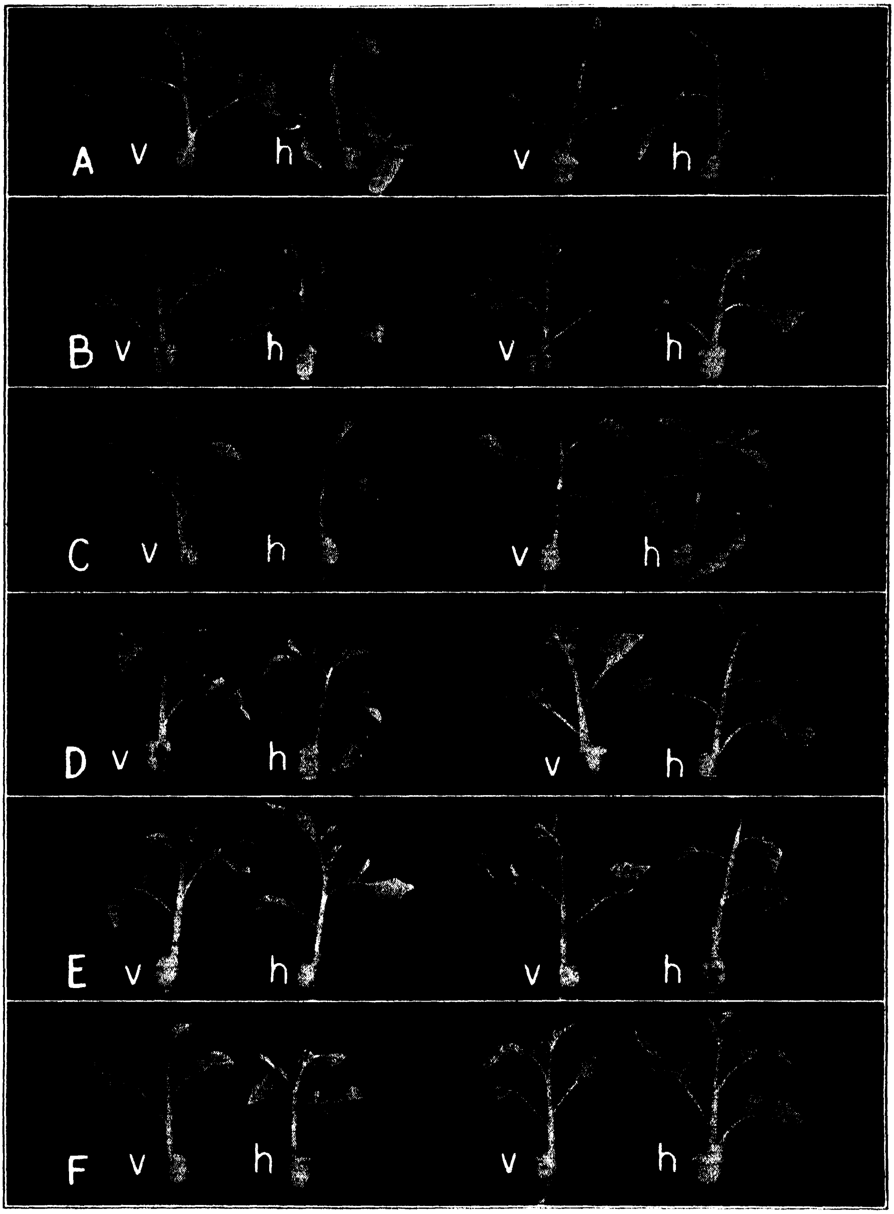


FIGURE 2. Effect of quantity of tomato stems and position toward gravity in producing an emanation causing epinasty of potato petioles. Lot A, 16 g.; B, 11 g.; C, 7.5 g.; D, 5.0 g.; E, 2.0 g.; F, 1.0 g. of tomato stems. The letters "v" and "h" refer to the vertical and horizontal positions in which the tomato stems were placed for 48 hours before the air surrounding the tomato stems was brought into contact with the potato plants.

RESULTS

The relation of the gravity-position and amount of tissue to the production of an emanation causing leaf epinasty is shown in Figure 2. The volatile products were allowed to accumulate for 48 hours in the tubes containing the tomato stems in the two gravity-positions, vertical and horizontal, and aliquots of the air surrounding the tomato stems were adjusted at the time of transference so as to introduce per liter of air in the test container an amount of emanation corresponding to 16, 11, 7.5, 5.0, 2.0, and 1.0 g. of tomato stem tissue in each of the two positions.

The photographs show the paired lots, the plant corresponding to the vertical position being at the left in each case. It is seen that in the vertical position only when the amount of tissue was as much as 16 g. was there any evidence of even slight epinasty, whereas in the horizontal position

FIGURE 3. Potato plants exposed to vapors produced by tomato stems in the two gravity positions. Top row, vertical position; bottom row, horizontal. The plants are arranged in the order corresponding to the paired amounts of tomato stem tissue used. The aliquots of the air removed for each test represent the following amounts of tomato stem tissue, reading left to right: 8.5, 7.4, 8.0, 8.5, 7.4, 4.5, 5.3, 4.6, 4.9, 5.3, 4.6, 4.0 grams.

good responses were obtained with 16, 11, 7.5, and 5 g. The results of this experiment, together with those of other tests, indicate that the production of the volatile substance causing epinasty is about three times as great in the horizontal as in the vertical position.

The results of another test are shown in Figure 3. In this case the amounts of tomato stem tissue represented in each pair varied only from 4.0 to 8.5 g. per liter of air space in the container. The top row shows the plants exposed to emanations produced in the vertical position and those in the lower row in the horizontal position. The plants are arranged in the proper order left to right to show the paired lots originally carefully selected to be equal in the number, weight, and length of tomato stems

used. Figure 3 shows an epinastic response from the horizontally-placed stems but not from those in the vertical position.

From the foregoing it should not be concluded that the emanation is not produced by stems in the vertical position. If the amount of tissue taken was large enough, epinasty was produced by stems in both positions, although in such cases, if there was an observable difference between the two lots the curvature produced by the horizontal position was induced earlier or the amount of curvature was greater. On the other hand in many cases the amount of tissue taken was inadequate to cause epinasty when stems were placed in either direction.

Combining all of the 151 paired tests that were made in 28 experiments from June 1935 to March 1936 the following results were obtained: horizontal +, vertical −, 85 cases; both positions +, but with the horizontal lots causing epinasty earlier, or giving a greater angle of curvature, 20 cases; both positions +, and with no differences observable, 12 cases; both positions −, 34 cases. In no case with paired lots of tissue was epinasty caused by the vertical position and at the same time not caused by the horizontal.

DISCUSSION

In these tests the tomato stems placed horizontal to gravity could not respond by bending so as to bring the stems toward the vertical position because the stems were fastened at both ends to the glass rod. The physiological conditions induced in the stems by the position toward gravity were maintained throughout the period of storage in the containers.

These results furnish further evidence connecting this volatile product with physiological processes in the plant producing it.

The experiments of Elmer (4), Kidd and West (7), and Gane (6) indicate that the production of this substance is particularly significant in the ripening stage of the apple fruit. Nelson and Harvey (8) found that it is given off by celery leaves only during the process of blanching. Zimmerman and Wilcoxon (9) found that treating plants with indoleacetic acid caused an increase in the capacity of the plant to induce epinasty of test plants placed in the surrounding air, due, they suggest, to the general increase in metabolism brought about by the auxin. In the present experiments the production of the emanation is shown to be related to still another physiological process: the response of stems to the force of gravity.

SUMMARY

The horizontal position toward gravity was found to be more effective than the vertical in causing the production by tomato stems of volatile products inducing epinasty of potato leaves in a manner similar to that resulting from low concentrations of ethylene.

LITERATURE CITED

1. BOTJES, JE. OORTWIJN. Aethyleen als vermoedelijke oorzaak van de groeiremmende werking van rijpe appels. Tijdschr. Plantenziek. **39**: 207–211. 1933.
2. DENNY, F. E. Testing plant tissue for emanations causing leaf epinasty. Contrib. Boyce Thompson Inst. **7**: 341–347. 1935.
3. DENNY, F. E., and LAWRENCE P. MILLER. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. **7**: 97–102. 1935.
4. ELMER, O. H. Growth inhibition of potato sprouts by the volatile products of apples. Science **75**: 193. 1932.
5. GANE, R. Production of ethylene by some ripening fruits. Nature [London] **134**: 1008. 1934.
6. ——— The formation of ethylene by plant tissues, and its significance in the ripening of fruits. Jour. Pomol. & Hort. Sci. **13**: 351–358. 1935.
7. KIDD, F., and C. WEST. The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Great Britain Dept. Sci. & Indus. Res., Food Invest. Bd. Rept. **1933**: 51–57. 1934.
8. NELSON, R. C., and R. B. HARVEY. The presence in self-blanching celery of unsaturated compounds with physiological action similar to ethylene. Science **82**: 133–134. 1935.
9. ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

SEVERAL ESTERS AS PLANT HORMONES

P. W. Zimmerman, A. E. Hitchcock, and Frank Wilcoxon

Much interest has been shown in recent reports of synthetic growth substances which induce responses similar to natural plant hormones. Theories concerning specificity of hormones lose support as new growth substances are located. As late as May 1935 F. W. Went (8, p. 166–167) wrote, "Kögl and co-workers have isolated three different crystalline substances, all giving positive reaction in the *Avena* and pea test, and, therefore, being growth-substances; physiologically they cannot be distinguished. They have been named auxin *a* ($C_{18}H_{32}O_5$); auxin *b* ($C_{18}H_{30}O_4$); and hetero-auxin (β-indolyl-acetic acid, $C_{10}H_9O_2N$); and all three of them are monobasic acids of about the same strength and have one double bond. . . . No other pure substances have been found to have any direct effect on growth at all; if saliva, diastase or other products have a growth accelerating effect, this is due to traces of one of the auxins. . . . From corn-germ-oil as well as from malt crystalline auxin *a* and *b* can both be prepared. So it seems probable that in the higher plants auxin *a* (and perhaps auxin *b*) are formed as *the* growth-substance. In the lower plants Kögl . . . could prove that hetero-auxin is formed, and not auxin *a* or *b*, . . . Rhizopin . . . must be identical with hetero-auxin."

During 1935 and 1936 (2, 10) there were reported from this laboratory thirteen acids and one nitrile which induced roots on stems and leaves and caused other responses similar to hetero-auxin which was tested in conjunction with the new substances.

In a recent report Söding (6) stated definitely that growth substances were non-specific.

The purpose of the present paper is to report nine esters of organic acids which accelerate growth locally, cause epinasty of leaves, bending of stems, and proliferations, and induce adventitious roots to form. All of these responses were similar to those induced by hetero-auxin and other synthetic growth substances previously reported. One of the esters, methyl β-indoleacetate, is more effective for accelerating local growth on green plants than hetero-auxin.

METHODS AND MATERIALS

To determine the comparative effectiveness of the esters with their corresponding acids, lanolin preparations with known amounts of the substances were applied by means of a glass rod to one side of the stem and to the upper side of tomato petioles. The plants used were similar in size and the petioles treated were selected for uniformity as to age and position on the stem. When high concentrations (0.05 per cent of methyl indole-

propionate) of the substances were used the petioles moved downward nearly paralleling the stem. In the lower limits of effective concentrations, the bending varied with the substances and the concentration. The exact effectiveness could be determined by measuring the angle between the petiole of the leaf and the stem before the substance was applied and then again after the response was complete. Bending occurred within two hours but the records were usually taken 10 to 18 hours after the preparation was applied. Water solutions of the esters were injected with glass tubes drawn to a capillary at one end or added to the soil. In the latter case 10 mg. per 50 cc. of water were used.

The following plants have been used as test objects: tomato (*Lycopersicon esculentum* Mill.), sunflower (*Helianthus debilis* Nutt.), Jerusalem artichoke (*Helianthus tuberosus* L.), *Kalanchoe daigremontiana*, and tropical grape (*Cissus sicyoides* L. var. *Jacquini* Planchon).

The following esters have been tested and found effective: *Naphthalene compounds:* methyl α-naphthaleneacetate, ethyl α-naphthaleneacetate. *Phenyl compounds:* methyl phenylacetate, ethyl phenylacetate, *n*-butyl phenylacetate, iso-butyl phenylacetate. *Indole compounds:* methyl β-indoleacetate, methyl β-indolepropionate, methyl β-indolebutyrate.

The naphthalene compounds were prepared from α-naphthaleneacetic acid in the Boyce Thompson Institute laboratories. Dr. R. H. Manske, National Research Council, Ottawa, Canada, supplied the indole compounds. The phenyl compounds were purchased from Eastman Kodak Co., Rochester, New York.

RESULTS

With comparable concentrations the methyl esters were more effective than the ethyl homologues, and the ethyl ester of the phenyl compounds was more effective than the butyl. The ethyl esters of the indole group have not yet been tested. It is probable, however, as indicated from results to date, that the methyl esters in all cases will be found more effective than any of the higher homologues of a given group of compounds.

The effectiveness of the esters compared with their corresponding acids was not of the same order for the different groups of substances. The methyl esters of all the indoles caused a greater bending response of tomato leaves and stems than similar concentrations of their corresponding acids (Fig. 1). With the naphthalene and phenyl compounds, the acids were found to be slightly more effective than their corresponding methyl esters.

Figure 1 is a photographic illustration of one set of results 17 hours after tomato leaf petioles and a portion of the stem had been treated with lanolin preparations containing comparable concentrations of indolepropionic acid and indolepropionate. The degree of bending and likewise the angle between the leaf and the stem varied with the concentration of

the substances. It can be seen that acceleration of growth was less and less as the substances were diluted down to the point where the lowest concentration of the acid did not cause a measurable response.

Results similar to those illustrated in Figure 1 were obtained when indoleacetic acid was compared with methyl indoleacetate and also when indolebutyric acid was compared with methyl indolebutyrate.

FIGURE 1. Tomato plants, showing the comparative effectiveness of indolepropionic acid and methyl indolepropionate for accelerating growth. Photographs were taken 17 hours after the plants had been treated. A, plants treated with lanolin preparations of the acid. Left to right: control, 0.05 per cent, 0.025 per cent, 0.01 per cent. B, plants treated with lanolin preparations of the ester. Left to right: control, 0.05 per cent, 0.025 per cent, 0.01 per cent. Note that in the lowest concentration the acid failed to induce bending.

The following figures show the lowest concentrations of the acids and their corresponding esters found to induce a measurable response on tomato leaves and the adjoining stem:

	Per cent in lanolin
β-indoleacetic acid	0.00125
Methyl β-indoleacetate	0.0006
β-indolepropionic acid	0.025
Methyl β-indolepropionate	0.01
β-indolebutyric acid	0.025
Methyl β-indolebutyrate	0.01

α-naphthaleneacetic acid	0.0025
Methyl α-naphthaleneacetate	0.005
Ethyl α-naphthaleneacetate	0.01
Phenylacetic acid	0.05
Methyl phenylacetate	0.1
Ethyl phenylacetate	0.2
n-butyl phenylacetate	0.5
Iso-butyl phenylacetate	1.0

With concentrations as high as 0.1 per cent of the indole (except indolebutyric acid) and naphthalene compounds, the bending response was so pronounced that the effectiveness of the acids and methyl esters could hardly be distinguished. To make fair comparisons, therefore, it was necessary to use a series of dilutions in the lower limits of effective concentrations (Fig. 1).

For initiating roots the order of effectiveness was approximately the same as for bending though higher concentrations of the substances were required. Very little difference, however, could be detected when optimum concentrations of the acids and their corresponding methyl esters were compared. The following range of concentrations of several substances was found effective for inducing roots on tomato stems and leaves:

	Per cent in lanolin
α-naphthaleneacetic acid	0.05–1.0
Methyl α-naphthaleneacetate	0.05–1.0
Ethyl α-naphthaleneacetate	0.10–1.0
β-indoleacetic acid	0.50–1.0
Methyl β-indoleacetate	0.50–1.0
β-indolepropionic acid	1.00–2.0
Methyl β-indolepropionate	1.00–2.0

Neither the upper nor lower limits of concentrations for inducing roots were determined. The number of roots induced from a given type of tissue varied with the concentration of the different substances.

Figure 2 A illustrates root induction on a stem of *Kalanchoe* from treatment with 0.5 per cent of methyl indoleacetate in lanolin.

Figure 2 B shows the similarity in rooting response induced by 1.0 per cent of the ethyl ester and 0.5 per cent of the corresponding acid of the naphthalene compounds. There were no qualitative differences observable in the character of roots produced by acids and esters. The roots shown in the illustration grew while the plants were standing on an open bench in the greenhouse. Had the tomato plants been placed in an environment with high humidity, the roots would have grown long and white. *Kalan-*

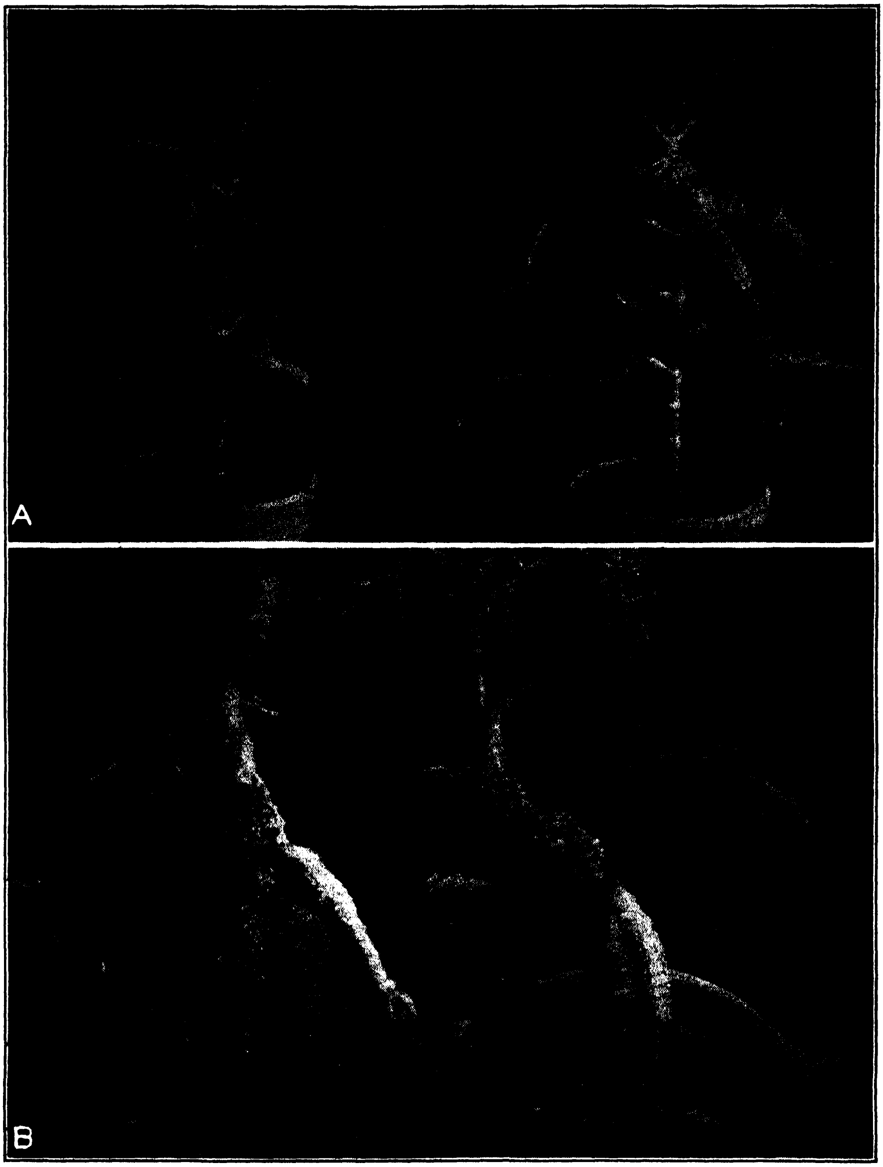


FIGURE 2. The root-inducing capacity of esters. A, *Kalanchoe daigremontiana*. Left, control. Right, plant showing roots induced by lanolin preparation containing 0.05 per cent methyl indoleacetate. Plants were kept continually on the greenhouse bench where the air was reasonably dry. Photograph taken 10 days after treatment. B, tomato plants, showing roots grown in dry air of the greenhouse after treatment with lanolin preparations of the growth substances. Left, leaf and one side of the stem above treated with 0.50 per cent α-naphthaleneacetic acid. Right, similar plant treated with 1.00 per cent ethyl α-naphthaleneacetate. Photograph taken 18 days after treatment.

choe, shown in Figure 2 A, produces white roots while growing in dry atmosphere. For this reason it should be a desirable plant type for teachers who wish to conduct experimental work with growth substances in dry laboratory air.

Aerial roots of *Cissus* (9) were more sensitive to the esters than stems or leaves of plants. When treated with effective lanolin preparation in the neighborhood of the tip elongation was practically stopped and branch roots were induced within three to five days. Concentrations of methyl naphthaleneacetate as low as 0.05 per cent of lanolin applied near the tip retarded elongation and induced new roots to form. Higher concentrations of the indole compounds were required for root induction.

The treated roots always bent toward the side where the substance was applied in contrast to stems and leaves. So far, no growth substances have been found to accelerate elongation in root tissue. This peculiar response of roots raises the question if there might exist in nature an entirely different type of natural hormone effecting elongation of the cells. For the root induction the substances seem to act the same on both stems and roots. For stems, however, the root-inducing substances are also the cell extension substances.

Water solutions of the esters were effective when injected into the stem or when added to the soil. Translocation of the substance was evidenced by the bending responses of stems and leaves. When the environmental conditions induced a high rate of transpiration the substances moved rapidly from the soil up the stem and out into leaves as described in an earlier report (3). Responses occurred within two hours after 50 cc. of water containing 10 milligrams of methyl indolepropionate was added to the soil. Due to the shortage of material no attempt was made to determine the range of effective concentrations. Ten milligrams of the esters per 50 cc. of water added to the soil of four-inch pots with six-inch tomato plants were found effective for methyl naphthaleneacetate, ethyl naphthaleneacetate, methyl indoleacetate, methyl indolepropionate, methyl phenylacetate, ethyl phenylacetate, and butyl phenylacetate. With the concentration given above, methyl indolepropionate induced the most pronounced response on aerial parts of the plants.

DISCUSSION

Shortly after it was reported (2, 5, 10) that thirteen different substances induced roots and caused other responses similar to those produced by use of hetero-auxin, Haagen Smit and Went (1) and Thimann (7) stated that the work was open to criticism because the experiments had been carried out on green, hormone-rich material in light and that the acid effect of the compounds, which might interfere with the transport of the growth hormones already in the plant, had not been considered.

Repeated attempts to induce responses described in previous papers by use of a variety of acids, many of which were closely related homologues of those called growth substances, resulted in failure. Also the concentrations of the substances in water solutions were so low that they could hardly affect the acidity of the treated tissue. At best they are weak organic acids and when diluted so as to contain only one part of the compounds to a million parts of water the acid effect on naturally buffered plant tissue is probably negligible.

The substances reported in the present paper are not acids but esters and their effectiveness should at once discount the theories concerning "acid effects." Also methyl β-indoleacetate is more effective than hetero-auxin (β-indoleacetic acid). The esters may be hydrolyzed to acids by the plant tissues, but there is no evidence at hand that such reactions occur. Neither do we know whether the so-called growth substances or auxins act directly or otherwise. Of most importance is the ultimate result—initiation of organs, and retardation or acceleration of growth. Only a few groups of compounds have been found to have the capacity to induce such responses in plants and it is unlikely that such important formative effects could be brought about merely by acids.

Kögl (4) once tested and compared the effect of methyl β-indoleacetate with that of β-indoleacetic acid for accelerating growth of oat coleoptiles. He reported the ester only two-fifths as effective as the acid. From later reports it appears that the esters have not been considered in a class with growth substances.

The data reported in this paper show that esters of the indole compounds are more effective for accelerating local growth of green plants than their corresponding acids. This is a matter of considerable importance since by Dr. Manske's method of manufacturing the indole acids, the esters are intermediary products.

The theories of specificity for natural hormones in plants must lose ground when other compounds as methyl β-indoleacetate are found to be more effective than hetero-auxin. Söding's (6) conclusion that growth substances are non-specific is quite in order.

SUMMARY

Nine esters have been added to the list of growth substances as follows: methyl α-naphthaleneacetate, ethyl α-naphthaleneacetate, methyl phenylacetate, ethyl phenylacetate, *n*-butyl phenylacetate, iso-butyl phenylacetate, methyl β-indoleacetate, methyl β-indolepropionate, methyl β-indolebutyrate. Methyl esters of these compounds were shown to induce adventitious roots on stems, leaves, and roots and to cause local acceleration of growth and other responses characteristic of growth substances.

One of the esters, methyl β-indoleacetate is more effective than heteroauxin (β-indoleacetic acid).

The methyl esters of the indole compounds were more effective for inducing bending responses than their corresponding acids. The methyl esters of the naphthalene and phenyl compounds were slightly less effective than the corresponding acids.

The methyl esters were more effective than the ethyl and the latter more effective than butyl esters.

The esters were taken up by the roots from water solutions added to the soil and transported upward as evidenced by formative responses of aerial parts of the plants.

LITERATURE CITED

1. HAAGEN SMIT, A. J., and F. W. WENT. A physiological analysis of the growth substance. Proc. Akad. Wetensch. Amsterdam, Sec. Sci. **38**: 852–857. 1935.
2. HITCHCOCK, A. E. Indole-3-*n*-propionic acid as a growth hormone and the quantitative measurement of plant response. Contrib. Boyce Thompson Inst. **7**: 87–95. 1935.
3. HITCHCOCK, A. E., and P. W. ZIMMERMAN. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. **7**: 447–476. 1935.
4. KÖGL, FRITZ. Über Wuchsstoffe der Auxin- und der Bios-Gruppe. Ber. Deutsch. Chem. Ges. **68**: A*16–28*. 1935.
5. MANSKE, RICHARD H. F., and LEONARD CHRISTIE LEITCH. The synthesis of δ-(3-indolyl)-valeric acid and the effects of some indol acids on plants. Canadian Jour. Res. **14**: Sec. B 1–5. 1936.
6. SÖDING, HANS. Wirkt der Wuchsstoff artspezifisch? Jahrb. Wiss. Bot. **82**: 534–554. 1936.
7. THIMANN, KENNETH V. On an analysis of the activity of two growth-promoting substances on plant tissues. Proc. Akad. Wetensch. Amsterdam, Sec. Sci. **38**: 896–912. 1935.
8. WENT, F. W. Auxin, the plant growth-hormone. Bot. Rev. **1**: 162–182. 1935.
9. ZIMMERMAN, P. W., and A. E. HITCHCOCK. The response of roots to "root-forming" substances. Contrib. Boyce Thompson Inst. **7**: 439–445. 1935.
10. ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

INCUBATION PERIOD OF PEACH YELLOWS IN ITS INSECT VECTOR

ALBERT HARTZELL

The incubation period required by the virus of a disease in its insect vector is interpreted by some as evidence that an obligate relationship exists between the virus and the insect involved. As yet there is no direct evidence that there is either multiplication of the virus or that the virus completes a cycle in the insect. Peach yellows is usually classified as a virus disease because of certain similarities between it and the mosaic diseases, notably that it is transmitted by grafting, budding, and tissue implanting, and that no organism has been found associated with it. Aside from insect transmission, this is the only means known. Unlike the viruses of mosaic diseases it has not been transmitted by needle inoculations. For convenience in this paper, it will be referred to as a virus disease. The term, incubation period of the virus in the insect, as used here, refers to the delay in the development of infective power within the insect. A number of instances are known in which a specific insect vector of a disease is incapable of transmitting the disease to a healthy plant until a minimum time has elapsed after feeding on a diseased plant. These periods vary from four hours to ten days, depending on the virus and the insect involved.

Smith and Boncquet (16) found that the beet leafhopper, *Eutettix tenella* Baker (*E. tenellus*) is not merely a mechanical carrier of curly leaf, but that some development or change takes place in the body of the insect during the first few hours after it feeds on a diseased plant, and that a period of 24 hours, and not more than 48 hours, must elapse before the insect can transmit the virus of the disease. Severin (12, 13, 14) showed that the incubation period is affected by temperature and found that the minimum time required was four hours at a mean temperature of 100° F. It was noted (6), however, that leafhoppers that had an incubation period of from one to three days transmitted curly top to a considerably higher percentage of beet seedlings than those, for instance, that had only from four to ten hours. Various periods are reported by different investigators, due no doubt to the different temperature conditions under which they were obtained. Carsner and Stahl (2) report that the period varies from 21 hours and 45 minutes to 24 hours.

Storey (17) found the minimum incubation period of the virus of streak disease of maize in its leafhopper vector, *Cicadulina mbila* Naude, to be from 6 to 12 hours at 30° C.

Kunkel (7, 8, 9) found that the incubation period of aster yellows virus in the leafhopper, *Cicadula sexnotata* Fall., showed a minimum of ten days. The period, he states, is variable and may be as long as three weeks. The

usual period in the greenhouse at 70° to 75° F. is from 12 to 14 days. All stages of the insects are capable of transmitting the virus. The nymphs are normally incapable of transmitting the virus because the incubation period in the insect is usually longer than the time required for them to reach maturity. When nymphs were kept at low temperature, the nymphal period was extended and they were able to transmit the virus as the incubation period within the insect was completed. He found that the period was somewhat shorter in adults (usually six to ten days) than in the nymphs (two weeks or more). It is interesting to note that with this disease the minimum incubation period in the insect is approximately the same as the minimum incubation period of the virus within the plant.

Dobroscky (3) found that with the virus of cranberry false blossom there was evidence that an incubation period exists in its leafhopper vector, *Euscelis striatulus* Fall., but did not determine definitely the length of the period.

Bald and Samuel (1) report an incubation period of the virus of spotted wilt of tomato of from five to seven days in its thrips vector, *Frankliniella insularis*, under medium temperature conditions.

Elze (4) working with potato leaf roll and *Myzus persicae* Sulz. noted a delay in the development of the infective power of the insect of from 24 to 48 hours, while Kenneth M. Smith (15) found an incubation period in the same insect of 54 hours.

Linford (11) finds an incubation period of approximately ten days in *Thrips tabaci* for the virus of yellow-spot of pineapple.

Similar delays in the development of infective power have been observed in insect-borne virus diseases of animals. With yellow fever and the mosquito, *Stegomyia fasciata*, there is an incubation period of from 12 to 14 days. The effect of temperature is very marked. At 22° C. the period is from three to four weeks, while at 20° C. the virus is unable to develop. Similarly, if a mosquito capable of transmitting the virus is exposed to low temperature, it loses its power, but regains it if the temperature is raised (21). In dengue fever, the same mosquito has an incubation period of from 11 to 14 days. The incubation period for the virus of sand fly fever in its vector, *Phlebotomus papatasi*, is about seven days; for typhus fever in the louse, *Pediculus corporis*, from seven to ten days, while for trench fever in the same insect, a period of from 5 to 12 days has been reported.

Kunkel (10) found that with the virus of peach yellows there was evidence that there may be a long incubation period in the leafhopper, *Macropsis trimaculata* Fitch, its insect vector, but did not determine the length of the period.

The writer's previous (5) infection experiments, with *Macropsis trimaculata* as a carrier of peach yellows, indicated that the incubation period in the insect may not exceed 22 days for the nymphs and 32 days for the

adults. A precise determination of the period was not made at that time, however, as attention was centered on the ability of the insect to transmit the disease, rather than on a study of the incubation period within the insect. The frequent transfers that are necessary to determine this period would have seriously interfered with the transmission experiments, owing to the limited number of trees and insects available. The present paper is an attempt to supplement our knowledge on this subject.

PLAN OF INCUBATION EXPERIMENTS

Potted peach seedlings (*Prunus persica* [L.] Stokes) used in these experiments had been grown for two seasons in insect-proof cages, except during the winter months when they were placed in a cold greenhouse. They ranged in age from two to five years. During the growing season of 1935, from time to time trees were removed as needed to an adjacent greenhouse where they were placed immediately in cages for infection tests. The remaining trees in the supply cages served as checks. Both lots of trees were in vigorous growing condition. Care was exercised to keep all cages insect-proof.

The cages in which infections were made were sufficiently large to permit from two to four trees to be exposed at one time. They were tightly constructed with wooden frames and floors. The sides were made of panes of glass while the ends and tops were covered with wire screen (50 meshes per inch). The greenhouse was shaded by coating the glass with a layer of white paint. The temperature of the greenhouse ranged from 70° to 90° F.

Nymphs of *Macropsis trimaculata* were collected from wild plum trees, (*Prunus americana* Marsh.) growing locally. As there were no other plum or peach trees within a radius of about one-half mile of these wild plum trees, it seems unlikely that the nymphs, being wingless, could become infected accidentally. As an additional check, healthy peach seedlings were exposed in cages to nymphs and adults transferred directly from these wild plum trees. In no case did any of these trees become infected, (Table I, trees No. 1, 2, 6, 7, 11, 46–48) indicating that the insects used came from a virus-free source. Attention in this study was centered on leafhoppers that had become infected as nymphs while caged on yellowed peach trees which had been under observation for a year or more. It was considered inadvisable to place reliance on adults captured in the field, since their ability to fly would greatly increase the probability of chance infection.

The nymphs were infected by allowing them to feed for periods of 1, 4, 7 and 10 days while caged on yellowed peach seedlings. They were then transferred to healthy peach seedlings where they fed for periods of from 1 to 24 days. From 4 to 20 insects were used in each of these tests. In all except two exposures, one or more live individuals were removed at the end

TABLE I

INCUBATION PERIOD OF PEACH YELLOWS IN MACROPSIS TRIMACULATA

Tree No.	Tree age, yrs.	No. days insects exposed	No. insects		Approximate stage of insect at beginning of exposure*	Date tree exposed, 1935	No. days tree exposed	No. live insects at at end of exposure		Date disease recorded, 1936	Approximate maximum incubation period in insect, days
			Nymphs	Adults				Nymphs	Adults		
1, 2	4–5	0	19	0	I	June 7	7	12	0	—	—
3	4–5	7	6	0	I	June 7	7	2	0	—	—
4, 5	4–5	4	20	0	I	June 7	7	6	0	No. 5, Feb. 6	11
6, 7	4–5	0	25	0	—	June 11	3	0	10	—	—
8	4–5	4	6	0	I, II	June 11	3	4	0	—	—
9, 10	4–5	1	13	0	II	June 12	1	3	0	—	—
11	4–5	0	12	0	—	June 14	13	1	8	—	—
12	4–5	**	4	0	—	June 14	3	0	0	Feb. 6	10†
13, 14	4–5, 3	4	9	0	II	June 17	7	2	0	No. 15, Feb. 6	11
15, 16	4–5, 3	4	9	0	II	June 17	18	0	2	—	—
17	4–5	4	20	0	II, III	June 18	17	0	9	—	—
18	3–4	4	19	0	II, III	June 18	6	0	6	—	—
19, 20	3–4	4	20	0	III	June 21	10	0	11	No. 20, Feb. 6	14
21, 22	4–5, 3	4	20	0	III	June 21	10	0	8	—	—
23, 24	3–4	1	20	0	III, IV	June 21	1	0	0	—	—
25, 26	3–4	1	14	0	III, IV	June 22	1	9	1	—	—
27, 28	3–4	4	15	0	IV	June 24	21	0	2	No. 27, Feb. 6	25
29, 30	3–4	4	0	13	III, IV	June 24	21	0	11	—	—
31, 32	3–4	4	0	16	III, IV	June 24	14	0	11	No. 32, Feb. 6	18
33, 34	3–4	7	0	17	III, IV	June 26	7	0	8	—	—
35, 36	3–4	10	0	16	III	June 27	15	0	9	—	—
37	2–3	4	0	19	V	July 1	4	0	9	—	—
38, 39	2–3	10	0	20	III, IV	July 1	10	0	7	—	—
40, 41	2–3	7	0	14	Adult	July 3	7	0	7	—	—
42–44	2–3	‡	0	20	Adult	July 5	4	0	5	No. 43, Mar. 20	26
45	2–3	4	0	15	Adult	July 8	3	0	5	—	—
46–48	2–3	0	0	20	Adult	July 8	7	0	3	—	—
Average incubation period in insect											16

* Nymphal instars indicated by Roman numerals.

** Nymphs previously used on tree No. 8.

† Minimum incubation period 7 or 8 days.

‡ Adults reared from nymphs previously used on trees No. 15, 16, and 17.

of the period. In a number of cases the nymphs had transformed to adults during the time that they were allowed to feed on the healthy trees. To prevent any individuals from being overlooked because of concealment, at the end of each exposure the trees and the inside of the cages were sprayed thoroughly with a rotenone preparation.

Upon completion of these tests, the trees that had been exposed to *Macropsis trimaculata* were removed to insect-proof cages out-of-doors.

FIGURE 1. Symptoms of peach yellows. Healthy twig at left showing normal leaf development. At right, three twigs from diseased trees to which yellows was transmitted by *Macropsis trimaculata*. Note difference in size of leaves and shortening of internodes.

After heavy frosts assured that there was little or no possibility of chance infection, both checks and treated trees were transferred to a cold greenhouse. On January 17th, 1936, all the trees were removed to a warm greenhouse to await development of the symptoms of peach yellows.

RESULTS OF INCUBATION TESTS

The results of the incubation tests with *Macropsis trimaculata* during the season of 1935, are shown in Table I and Figure 1. It will be noted that of 48 trees exposed to infected leafhoppers, 7 became diseased or 14.6 per cent. The maximum incubation period ranged from approximately 10 days to 26 days. In one case, adults infected as nymphs succeeded in transmit-

ting the disease to a healthy peach seedling. The incubation period within the insect was approximately 26 days in this instance. Since the insects were not moved to different trees daily, owing to lack of facilities for handling such a large number of trees, it is not possible to define the incubation period within the insect closer than stated above. The method, therefore, was better suited for determining the maximum incubation period rather than the minimum. In one instance where a tree (Tree No. 12, Table I) that was given short exposures to infective insects became diseased, the period could not have been less than seven or eight days. The average period was approximately 16 days. Nine peach seedlings (Trees No. 1, 2, 6, 7, 11, 46–48, Table I) exposed to non-infected leafhoppers and 38 trees that were not exposed to insects, which were held as checks, remained healthy. Records of a number of trees that were exposed to insects, but did not survive the winter, are not included in the table.

Nymphs and adults that were allowed to feed for four days on yellowed peach seedlings were capable of picking up the virus and transferring it to healthy trees. The few trials with leafhoppers allowed to feed for periods of 1, 7 and 10 days did not result in infections. This failure to transmit the disease may have been due to chance rather than any definite period required. Although in a number of cases the leafhoppers were used in more than one test, no evidence was obtained on the longevity of the virus within the insect vector.

The incubation period of the virus within the peach tree, while long, did not exceed previous records for this disease.

In general, the results of the incubation tests are similar to those obtained by Kunkel (8) for *Cicadula sexnotata* with aster yellows, but the period is somewhat shorter than was found for nymphs of that species.

DISCUSSION

Two views may be held regarding the incubation period of the virus in the insect vector. The incubation period may represent either a mechanical or a biological internal transmission in which the infective agent develops and multiplies within the insect's body or the time required for the virus to pass down the alimentary canal, diffuse through the intestinal wall into the blood stream, and return to the saliva with which it passes out into the plant host during the process of feeding. The leafhoppers possess an esophageal valve which prevents regurgitation of virus-containing sap. Attempts by Swezy (20) and Kenneth M. Smith (15) to correlate the movement of virus by the use of dyes resulted negatively.

By artificial inoculation, Storey (19) was able to render *Cicadulina mbila* and *C. zea* infective to streak of maize. Inactive races of these species were made active by needle inoculation with the juice of diseased plants. Also a single puncture of the abdomen with a virus-free needle in some cases

caused the leafhoppers to become active provided they fed before or after on diseased plants. This treatment was successful only if punctures were made through some part of the intestine. He concludes that the virus entering the intestine by mouth passes through the intestinal wall into the blood, and that in an inactive insect the cells of the intestinal wall resist the passage of the virus. Once the barrier of the intestinal wall is passed, the virus behaves as in an active insect.

Whether the failure of *Macropsis trimaculata* to transmit peach yellows constantly is due to the failure of certain individual leafhoppers to pick up the virus or to the presence of an inactive race was not determined in the present investigation. Recently Storey (18) has bred two races of *Cicadulina mbila*, one capable of transmitting the virus of streak disease, and the other unable to transmit the disease. The presence of active and inactive races of *Macropsis trimaculata* is suggested as a possible explanation of this phenomenon.

SUMMARY

The incubation period of the virus of peach yellows in its insect vector, *Macropsis trimaculata*, was determined approximately under greenhouse conditions. Since the interval between successive transfers of the individual insects from tree to tree was necessarily comparatively long, a very precise determination of the period cannot be made.

Nymphs were allowed to feed on diseased seedling peach trees for periods of 1, 4, 7 and 10 days prior to being placed on healthy trees. Nymphs of the 4-day series only became infective.

The maximum incubation period of the virus of peach yellows as experimentally determined was found to range from 10 to 26 days, with an average of 16 days. The minimum period in one instance was from 7 to 8 days. All positive infections were obtained with insects that had become infected as nymphs and were allowed to feed on healthy trees during the nymphal stage, except in one instance in which the leafhoppers had transformed to adults while feeding on healthy trees during successive transfers. The maximum period in this case was 26 days.

LITERATURE CITED

1. BALD, J. G., and GEOFFREY SAMUEL. Investigations on "spotted wilt" of tomatoes. II. Australia Coun. Sci. & Indus. Res. Bull. 54. 24 pp. 1931.
2. CARSNER, EUBANKS, and C. F. STAHL. Studies on curly-top disease of the sugar beet. Jour. Agric. Res. **28**: 297–320. 1924.
3. DOBROSCKY, IRENE D. Studies on cranberry false blossom disease and its insect vector. Contrib. Boyce Thompson Inst. **3**: 59–83. 1931.
4. ELZE, D. L. De verspreiding van virusziekten van de aardappel (*Solanum tuberosum* L.) door insekten. Mededeel. Landbouwhoogesch. [Wageningen] **31**(2): 1–90. 1927.
5. HARTZELL, ALBERT. A study of peach yellows and its insect vector. Contrib. Boyce Thompson Inst. **7**: 183–207. 1935.

6. Incubation period. *In* Entomology and parasitology. California Agric. Exp. Sta. Ann. Rept. **1922–23**: 127–128. 1923.
7. KUNKEL, L. O. Incubation period of aster yellows in its insect host. Phytopath. **16**: 67. 1926.
8. ——— Studies on aster yellows. Amer. Jour. Bot. **13**: 646–705. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 181–240. 1926.)
9. ——— The aster yellows disease. Proc. 4th Internat. Congr. Plant Sci. Ithaca [New York] 1926: 1249–1253. 1929.
10. ——— Insect transmission of peach yellows. Contrib. Boyce Thompson Inst. **5**: 19–28. 1933.
11. LINFORD, MAURICE B. Transmission of the pineapple yellow-spot virus by *Thrips tabaci*. Phytopath. **22**: 301–324. 1932.
12. SEVERIN, HENRY H. P. Minimum incubation periods of causative agent of curly leaf in beet leafhopper and sugar beet. Phytopath. **11**: 424–429. 1921.
13. ——— Curly leaf transmission experiments with beet leafhopper (*Eutettix tenella* Baker). Jour. Econ. Ent. **15**: 182. 1922.
14. ——— Minimum incubation periods of causative agent of curly leaf in beet leafhopper (*Eutettix tenella* Baker) and sugar beet. Phytopath. **12**: 105. 1922.
15. SMITH, KENNETH M. Studies on potato virus disease. IX. Some further experiments on the insect transmission of potato leaf-roll. Ann. App. Biol. **18**: 141–157. 1931.
16. SMITH, RALPH E., and P. A. BONCQUET. Connection of bacterial organism with curly leaf of sugar beet. Phytopath. **5**: 335–342. 1915.
17. STOREY, H. H. Transmission studies of maize streak disease. Ann. App. Biol. **15**: 1–25. 1928.
18. ——— The inheritance by an insect vector of the ability to transmit a plant virus. Proc. Roy. Soc. [Lond.] B. **112**: 46–60. 1932.
19. ——— Investigations of the mechanism of the transmission of plant viruses by insect vectors. I. Proc. Roy. Soc. [Lond.] B. **113**: 463–485. 1933.
20. SWEZY, OLIVE. Factors influencing the minimum incubation periods of curly top in the beet leaf hopper. Phytopath. **20**: 93–100. 1930.
21. A system of bacteriology. vol. 7. 509 pp. Medical Research Council, Publ. by His Majesty's Stationery Office, London. 1930.

RELATION BETWEEN QUANTITY OF ETHYLENE CHLORHYDRIN ABSORBED AND GROWTH RESPONSE IN TREATMENTS FOR SHORTENING THE REST PERIOD OF POTATO TUBERS[1]

LAWRENCE P. MILLER AND F. E. DENNY

Experiments conducted over a period of years in this and other laboratories (1, 2, 3, 4, 6, 7) have shown that the rest period of potato tubers (*Solanum tuberosum* L.) is broken by exposing the whole tubers to the vapor of ethylene chlorhydrin. The treatments are made by placing the tubers together with some of the liquid chemical added to cheesecloth in a closed container for from 24 hours to several days. The vapor pressure of chlorhydrin at the temperatures used has been determined (4) and it has been found that the quantities of chlorhydrin that have been shown to be favorable for shortening the rest period are far in excess of the amounts necessary to saturate the air space in the containers used for the treatments. This would indicate that the tubers take up appreciable quantities of chlorhydrin and that differences in the physiological responses that have been noted when a series of treatments are made in which the concentration of chlorhydrin available varies over a considerable range, are due to differences in the amounts of chlorhydrin taken up by the tubers and not to any important differences in the concentration of the chemical maintained as a vapor around the tubers during the treatment period, since the quantities of chlorhydrin present in all cases are more than enough to saturate the air space used.

In the present paper are reported investigations in which dormant tubers were subjected to the vapor of ethylene chlorhydrin under a variety of conditions and the response studied from the point of view of its relation to the quantity of chlorhydrin taken up by the tubers during the exposure. The experiments were so designed as to permit the determination of the minimum concentration of chlorhydrin in the tubers which would suffice to overcome the rest period, and the maximum concentration which could be tolerated without injury. Studies were also made of the factors influencing the absorption of chlorhydrin by the tubers.

METHODS

The methods employed in exposing the tubers to the vapor of the chemical were similar to those previously used and are described more fully in connection with the individual experiments. For the determination of the chlorhydrin absorbed by the tubers the method given in an earlier

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 113.

paper was followed except for a few minor changes (4). Samples of the treated tubers weighing 300 grams were cut into pieces of about two grams each and placed in two-liter Florence flasks, together with 500 cc. of distilled water and 10 cc. of N H_2SO_4. (The reason for the addition of the acid was to counteract the increase in the pH of the tubers which results from treatment with chlorhydrin.) The mixture was then carefully distilled (care must be taken to prevent excessive foaming) and 400 cc. of distillate collected in two 200-cc. volumetric flasks containing 4.5 g. of solid $Ba(OH)_2$ per 100 cc. These solutions were allowed to stand overnight after which aliquots were taken which were acidified with nitric acid, treated with an excess of N/20 $AgNO_3$ and back titrated with N/20 KSCN, using ferric ammonium sulphate as an indicator. In the tests with single tubers, samples weighing from about 25 to 100 grams were distilled in one-liter flasks after the addition of 2 cc. of N H_2SO_4 and 275 cc. of H_2O. Two hundred cc. of distillate were collected. In each case a Kjeldahl connecting bulb was inserted between the flasks and the condensers.

RESULTS

MINIMUM CONCENTRATION REQUIRED AND MAXIMUM CONCENTRATION TOLERATED BY THE TUBERS

In order to determine the minimum quantity of chlorhydrin effective in shortening the rest period and the maximum quantity which could be tolerated without injury, dormant potato tubers were subjected to the vapor of ethylene chlorhydrin under conditions in which the quantity of chemical absorbed varied over a wide range. Experiments were conducted with three lots of potatoes, tubers of the Irish Cobbler variety obtained from South Carolina in the spring of 1935 and tubers of the Irish Cobbler and Bliss Triumph varieties grown in the Institute gardens in the summer of 1935. The treatments were made in tall cylindrical galvanized tanks of 800-liter capacity. The chlorhydrin was added to cheesecloth placed on a piece of wire gauze suspended about 18 inches from the top of the tanks under a 12-inch blade from an electric fan which was rotated in order to mix the atmosphere within the containers during the treatment period. A mercury seal was used to make the container air-tight at the point where the shaft carrying the fan blade entered. Some of the tests were run without stirring to test the effect of stirring on the amount of chlorhydrin absorbed. The tubers were placed in a wire basket which rested a few inches from the bottom of the containers. Seventy tubers were used in each test with the potatoes from South Carolina (except the last one in which only 39 tubers were treated), and in the experiments with the tubers from the Institute gardens wire baskets of about 8-liter capacity containing about 6000 g. of tubers were used. In each case a series of treatments was conducted at the same time with the quantity of chlor-

hydrin present in the containers varying over a considerable range. Quantities from 375 cc. to 14 cc. of the 40 per cent solution per container were used. The treatments were made at various intervals after harvest and at three different temperatures, 22°, 25°, and 30° C. Controls were subjected to the same temperature without being exposed to any chemical. The effect on growth was determined by planting 50 pieces with one eye each from each treatment immediately after the end of the treatment period and at intervals thereafter up to 16 days after the end of treatment. The pieces were planted in flats containing 25 pieces each and kept at room

TABLE I

RELATION BETWEEN QUANTITY OF CHLORHYDRIN ABSORBED AND GROWTH RESPONSE IN VAPOR TREATMENTS OF DORMANT IRISH COBBLER POTATOES (25° C.)

Treated, days after receipt	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers	Days for 50% to emerge*	% rot
3	159.6	—	100
3	97.3	14	70
10	26.3	7	8
10	19.0	8	8
19	18.9	5	2
3	16.4	10	0
27	11.9	9**	12
19	10.4	7	2
27	9.0	9**	16
19	4.3	6	4
10	4.0	6	0
27	3.2	11**	10
10	2.5	6	0
27	1.6	12	0
19	1.0	11	0
8–14	Control	50	3
15–21	"	26	1.5
22–28	"	31	0.4
29–35	"	11	1

* Counted from time of planting, 4 days after end of 5-day treatment. Percentages are based on number of pieces which germinated.

** Planted 8 days after end of treatment.

temperature. They were examined at intervals of about five days and any pieces showing sprouts above ground were discarded and the number recorded. In this way it was possible to determine the number of days required for a certain percentage of pieces to show sprouts above ground.

The results of the plantings made four days after the end of the treatment are shown in Tables I, II, and III. The treatments are listed in order of decreasing concentration of chlorhydrin recovered from the tubers immediately after treatment. Quantities recovered ranged from 0.1 to 159.6 cc. 0.1 molar solution per 100 grams of tissue. This is equivalent to from 0.5 cc. to 868 cc. of the 40 per cent solution per bushel (to convert cc.

0.1 molar per 100 g. to cc. 40 per cent per bushel multiply by 5.4). The maximum concentration tolerated without serious injury is about 15 cc. 0.1 molar solution per 100 g. tissue at 30°, 25 cc. 0.1 molar at 25°, and somewhat higher at 22° C. Concentrations less than 1.0 cc. 0.1 molar solu-

TABLE II

RELATION BETWEEN QUANTITY OF CHLORHYDRIN ABSORBED AND GROWTH RESPONSE IN VAPOR TREATMENTS OF DORMANT BLISS TRIUMPH POTATOES

Treated, days after harvest	Temp. of treatment, ° C.	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers	Germination record			
			Days for 50%*	Days for 80%*	Days for 92%*	% rot
3	30	139.0	—	—	—	100
28		73.9	—	—	—	100
10		58.0	13	18	22	44
25		47.2	18	27	35	0
3		24.8	—	—	—	100
10		13.4	8	10	13	4
18		7.7	16	29	35	60
3		5.5	6	10	11	14
25		2.2	14	21	32	0
18		1.5	9	14	17	4
10		1.2	6	8	9	0
25		0.3	15	29	35	0
2	22	145.7	23	28	36	78
2		50.7	13	18	21	8
2		49.8	11	15	17	12
17		25.1	11	16	23	20
9		17.0	9	13	15	0
9		14.3	10	13	15	2
2		13.8	8	12	14	0
17		13.4	9	11	15	4
24		11.8	12	16	18	0
24		10.6	12	15	16	0
17		7.1	6	9	10	0
24		6.9	10	15	17	0
9		5.0	9	12	14	0
17		2.8	7	9	10	6
9		2.1	8	11	13	0
24		0.8	14	16	21	0
7–13	Av. of controls at 22 and 30	Control	27	50	72	0
14–20			21	35	47	0
21–27			20	38	51	1
28–34			29	48	63	0.4
35–41			34	51	66	0.4
42–48			19	31	46	2

* From time of planting, 4 days after end of 5-day treatment period. Percentages are based on number of pieces which germinated.

tion per 100 grams were still effective in overcoming the rest period. With respect to shortening the rest period the lower concentrations are more effective at the higher temperatures. In studying the data from the viewpoint of breaking dormancy, special emphasis should be placed on the results of Table III, since the tubers used in the experiments reported in

this table were definitely more dormant than tubers in the other series.

In considering these values it must be borne in mind that the figures for the quantity of chlorhydrin recovered probably do not represent all

TABLE III

RELATION BETWEEN QUANTITY OF CHLORHYDRIN ABSORBED AND GROWTH RESPONSE IN VAPOR TREATMENTS OF DORMANT IRISH COBBLER POTATOES

Treated, days after harvest	Temp. of treatment, °C.	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers	Germination record			
			Days for 50%*	Days for 80%*	Days for 92%*	% rot
6	30	44.5	6	10	12	44
21		36.3	10	14	18	12
29		27.3	10	14	18	60
36		16.7	14	19	22	2
21		4.9	7	11	22	2
6		4.4	6	10	16	8
14		1.8	7	11	24	4
29		1.7	13	19	25	24
36		1.7	15	23	44	4
36		1.1	34	55	61	0
29		0.5	12	21	52	4
21		0.3	8	21	30	0
14		0.2	14	28	67	0
6		0.1	8	11	20	2
14		0.1	14	50	68	6
13	22	8.3	10	14	23	0
35		7.7	10	14	16	0
35		7.5	9	13	16	0
28		3.6	6	9	10	2
35		3.5	9	15	27	0
35		2.4	10	16	27	0
28		2.1	9	13	24	0
28		2.1	7	10	12	0
13		1.7	8	16	45	0
20		1.2	10	30	46	0
20		0.9	10	60	103	0
13		0.6	12	45	71	0
20		0.2	28	65	75	0
28		0.2	11	21	36	0
13		0.1	14	43	68	0
13		0.1	42	62	75	0
0–6	Av. of controls at 22 and 30	Control	73	100	115	0
7–13			84	100	116	0
14–20			75	91	105	0
21–27			54	81	93	0
28–34			58	79	88	0.3
35–41			56	73	87	0.3
42–48			57	74	89	0.3
49–57			41	58	67	0.8

* From time of planting, 4 days after end of 5-day treatment period. Percentages are based on number of pieces which germinated.

the chlorhydrin taken in. The method of distillation used in these determinations has been estimated to give only 85 per cent recovery (4, p. 175). Then too it is known that some chlorhydrin is decomposed in the

tubers in the course of time (4, p. 177). Since these treatments were all continued for five days a certain amount of chlorhydrin taken in was decomposed and no longer existed as unaltered chlorhydrin when the determinations were made. An attempt was made to determine the amount of chlorhydrin decomposed by determining the total chloride content of the tubers at the end of treatment and comparing these figures with the chloride accounted for by the chlorhydrin recovered, after making due correction for the chloride content of the controls. Since it is not practical to use large quantities of tissue for the determination of total chloride, samples of 25 to 40 grams were used and the results obtained were too variable, presumably because the samples were not sufficiently representative, to permit of an accurate estimate of the quantity of extra chloride present, over and above that accounted for by the chlorhydrin recovered. The results definitely show, however, that the tubers at the end of treatment contained extra chloride not accounted for by the chlorhydrin recovered even after correcting for 85 per cent yield. The results do not permit a statement of the actual amount decomposed with any great degree of accuracy but indicate that it is of the same order of magnitude as previously found by another method which showed that tubers decomposed chlorhydrin to the extent of from 0.3 to 0.9 mg. per 100 g. per hour (4).

If the rate of decomposition of the chlorhydrin within the tubers is not much different when larger amounts are present than when the amount taken up is small, the extra quantity taken in and not accounted for by the recovery by distillation at the end of treatment is more important on a percentage basis in cases in which only a small amount of chlorhydrin is recovered. This would tend to narrow somewhat the effective range as shown in Tables I, II, and III. Even with this in mind this range is still quite large. Thus, if the quantity of chlorhydrin added to a container for a five-day treatment period were equivalent to 20 cc. 0.1 molar solution per 100 g. this quantity would not cause injury in the case of treatments at 22° to 30° C., even if it were practically all absorbed, which would happen only if the tubers were treated within a week or ten days after harvest. If all the chlorhydrin available were absorbed the quantity recovered would probably be about 15 cc. 0.1 molar solution per 100 grams; 5 cc. are deducted to take care of the chlorhydrin decomposed during the treatment period and the lack of complete recovery by the distillation method. If only half of the available chlorhydrin were absorbed, which would more likely be the case, the amount recovered would be 5 cc. 0.1 molar solution per 100 g. This quantity according to the data in Tables I, II, and III would be a most desirable quantity at all the temperatures studied both from the viewpoint of efficacy in breaking dormancy and of freedom from any possibility of injury. Probably treatments with 10 cc.

0.1 molar solution per 100 grams, or even 5 cc., would be sufficient in all cases and somewhat safer with freshly-harvested tubers, especially if treated at temperatures above 25° C. The amounts considered here, 5 to 20 cc. 0.1 molar solution per 100 grams of potatoes, correspond to 27 tc 108 cc. of the 40 per cent solution per bushel of potatoes, or if treatments are made under conditions in which one-fourth of the space in the container is taken up by tubers and we consider a bushel to occupy 36 liters, these quantities correspond to 0.18 to 0.75 cc. per liter.

In the discussion above the quantity of chlorhydrin has been designated in terms of cc. 0.1 molar solution per 100 grams of tubers or cc. of the 40 per cent solution per bushel rather than in terms of cc. per liter of air space in the container, as has been the custom previously. In view of the fact that the recent work has shown that the tubers absorb relatively large quantities of the chemical the effective concentration cannot be expressed accurately in terms of cc. per liter of air space unless the ratio between the quantity of tubers used and the total air space is constant in all experiments. Since this is not usually the case and since the concentration attained within the tubers seems an important factor in the growth response, it is proposed that the concentration be expressed in terms of the tubers to be treated. Within certain limits then the size of the container is not important, and treatments in which the amount of tubers or size of container have varied can be compared.

Table IV summarizes some experiments in which the effect of varying the quantity of potatoes, size of container, and cc. of 40 per cent solution per liter have been studied. These tests show that the most important single relationship from the viewpoint of the amount of chemical taken up is the relation between the quantity of potatoes and the quantity of chlorhydrin. In the last series shown the quantity of chemical was increased without increasing the concentration attained within the tubers. This will be discussed further on in the paper in which the effect of the permeability of the skin is taken up. It is obvious, of course, that if the quantity of chlorhydrin present is more than can be absorbed by the tubers from a saturated atmosphere during the treatment period, the addition of more liquid chlorhydrin cannot increase this absorption.

In the experiments reported in Table IV, treatments were carried out at room temperature for 24-hour periods and the tubers were cut into pieces and planted immediately after the end of the treatment. The tubers used were from the same lot as were used for the tests shown in Tables II and III and the growth record of controls can be seen in these tables. The data are in general agreement with the results in Tables I, II, and III, with regard to the maximum concentration tolerated without injury. The effects on overcoming dormancy were not quite as good. These results confirm previous conclusions in which the efficacy of a longer period of

TABLE IV

EFFECT OF VARYING THE QUANTITY OF POTATOES, SIZE OF CONTAINER, AND AMOUNT OF CHLORHYDRIN PRESENT ON THE QUANTITY OF CHLORHYDRIN ABSORBED AND ON SUBSEQUENT GROWTH

Variety	Days after harvest	Amt. of tubers, grams	Size of container, liters	Cc. 40% chlorhydrin present	Cc. 0.1 M chlorhydrin absorbed	Growth record: Days for 50%*	Growth record: Days for 80%*	Growth record: Days for 92%*	Growth record: % rot
Irish Cobbler	7	500	1	0.5	3.8	63	94	101	0
		500	2	1.0	10.3	61	80	82	0
		500	4	2.0	15.7	52	68	77	0
		500	17.5	8.25	15.8	33	54	62	0
		500	75	37.5	53.7	12	22	49	15
Bliss Triumph	3	500	1	0.5	6.4	14	21	40	0
		500	2	1.0	9.0	13	24	28	0
		500	4	2.0	15.8	11	15	19	0
		500	8.5	4.25	28.6	16	24	28	0
		500	17.5	8.75	30.1	11	15	27	0
		500	30	15.0	64.4	15	19	22	10
		500	75	37.5	89.7	14	16	21	45
Bliss Triumph	9	500	1	0.5	5.3	13	16	19	0
		1000	2	1.0	3.2	13	18	24	0
		2000	4	2.0	4.8	14	16	17	0
		500	8.5	4.25	13.5	14	16	17	0
		1000	17.5	8.75	13.0	12	15	17	0
		2000	35	17.5	14.4	13	16	17	20
		4000	70	35.0	27.7	13	18	28	20
Irish Cobbler	12	500	1	5	16.8	22	52	55	5
		500	2	5	17.9	42	65	83	0
		500	4	10	21.0	42	60	68	0
		500	8.5	10	19.8	22	56	73	5
		500	17.5	10	8.5	65	83	90	0
		500	30	10	7.3	46	56	65	5
		500	75	10	4.4	58	77	83	0
Bliss Triumph	31	500	75	18.75	11.0	15	17	20	0
		500	75	37.5	16.7	16	21	23	0
		500	75	75.0	10.7	23	29	31	0
		500	75	150.0	9.9	16	28	34	0

* Counted from time of planting immediately after 24-hour treatment period. Percentages are based on number of pieces which germinated.

treatment and a short period of storage (several days) after treatment and before planting were stressed (2, 4).

FACTORS AFFECTING ABSORPTION

Once the favorable range of concentration of chlorhydrin to be attained within the tubers has been determined the next step is to study the most efficient means of introducing these concentrations. Since the treatments are conducted by placing the tubers within a closed container together with some liquid chlorhydrin, it follows that the two most im-

portant factors to be considered are the rate of vaporization of the chemical and the rate at which the tubers take up the chemical from the vapor surrounding them. If the rate of vaporization of the chemical were a limiting factor this could be increased in various ways such as increasing the temperature (the partial pressure of the chlorhydrin in the 40 per cent solution is 2.3 mm. mercury at 20° C. and 4.2 mm. at 30°) or by the mechanical circulation of the air within the container. Experiments with a number of different lots of tubers and under a variety of conditions, including containers of 75-liter and 800-liter capacity and duration of treatments ranging from 6 hours to 5 days, have shown that in general the amount of chlorhydrin taken up is not increased as a result of air circulation. Similarly, tests conducted on the effect of temperature have indicated that under some conditions tubers take up about the same quantity of chlorhydrin at different temperatures. These results indicate that the rate of vaporization is usually not limiting but that the permeability of the skin of the tubers is the controlling factor. Since the permeability of the tubers varies greatly, as will be shown below, it is possible to pick out special situations under which stirring or increased temperature hastens absorption. However, under the conditions usually prevailing when tubers are to be treated for shortening the rest period the rate of vaporization of the chemical is ample and need not be increased by stirring or by increasing the temperature.

Permeability of Intact Tubers

The freshly-harvested tubers take up much larger quantities of chlorhydrin when exposed under the same conditions than tubers from the same lot at a later date. This is shown in Table V which lists results of treatments with tubers of the Irish Cobbler variety obtained from South Carolina. The exact date of harvest of these tubers is not known but they were shipped promptly after harvest and time of treatment is indicated in Table V on the basis of number of days since the tubers were received. It is seen that in the early stages the amount of chlorhydrin taken up decreases rapidly with time after harvest, older tubers taking up only one-fifth or one-tenth of the quantity taken up by the freshly-harvested tubers under the same conditions of treatment. A similar result is shown quite strikingly in Table VI which gives the quantity of chlorhydrin taken up by the tubers of the Irish Cobbler and Bliss Triumph varieties at intervals after harvest. In this case the two varieties were treated together in the same container and therefore exposed to identical conditions with respect to the chlorhydrin vapor available, but the Bliss Triumph variety was harvested 11 days later than the Cobbler variety. It is seen that these 11 days exert a marked influence in the early stages after harvest so that the Bliss Triumph potatoes take up 50 and more times the quantity ab-

sorbed by the Irish Cobbler potatoes treated in the same container. Somewhat later, 10 days after harvest, the permeability of the Bliss Triumph variety has decreased to such an extent that the tubers of the Irish Cobbler variety actually took up more than in earlier treatments. This is not considered to be due to an increase in the permeability of the Irish Cobbler tubers but to the fact that the competition from the Bliss tubers which were adjacent has lessened. The quantity of chlorhydrin available for

TABLE V

EFFECT OF TIME SINCE HARVEST ON QUANTITY OF CHLORHYDRIN ABSORBED BY POTATO TUBERS WHEN EXPOSED TO THE VAPOR

Treated, days after receipt	No. of tubers in each treatment	Cc. 40% chlorhydrin per 800 liter for 5 days	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers
3	70	375 125 42	159.6 97.3 16.4
10	70	125* 125 42 14	26.3 19.0 4.0 2.5
19	70	125* 125 42 14	10.4 18.9 4.3 1.0
27	39	125* 125 42 14	11.9 9.0 3.2 1.6

* In the treatments marked thus the air was not stirred during treatment.

absorption was equivalent approximately to 100, 35, and 12 cc. 0.1 molar solution per 100 g. of tubers in the treatments with 125, 42, and 14 cc. of the 40 per cent solution per container. The data show that in the early treatments the Bliss Triumph potatoes took up such a large proportion of the total chlorhydrin present that little was available for the Irish Cobbler tubers. These results show that the permeability of the tubers is extremely high immediately after harvest and that it drops rapidly for the first week or ten days after which the further decrease resulting is much less rapid.

Because of this extremely extensive alteration of the ability of the tubers to take up chlorhydrin with time after harvest it is not possible to predict, even if the conditions of treatment are closely controlled, the quantity of chlorhydrin the tubers will take up as a result of treatment. This is evident also in the last series shown in Table IV, in which the

quantity of chlorhydrin available for absorption was relatively large compared with the other series shown in the table and with the experiments recorded in Tables V and VI. Due to the decrease in the quantity that the

TABLE VI

RELATIVE AMOUNTS OF CHLORHYDRIN ABSORBED BY TWO VARIETIES OF POTATOES WHEN TREATED AT THE SAME TIME IN THE SAME CONTAINERS

Days after harvest		Temp. of treatment, ° C.	Cc. 40% chlorhydrin per 800 liter for 5 days	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers	
Irish Cobbler	Bliss Triumph			Irish Cobbler	Bliss Triumph
6	—	30	125	44.5	—
			42	4.4	—
			14	0.07	—
13	2	22	125	1.7	145.7
			42	0.6	49.8
			42*	0.1	50.7
			14	0.1	13.8
14	3	30	125	1.8	139.0
			42	0.2	24.8
			14	0.1	5.5
20	9	22	125	8.3	14.3
			42	1.2	17.0
			42*	0.9	5.0
			14	0.2	2.1
21	10	30	125	36.3	58.0
			42	4.9	13.4
			14	0.3	1.2
28	17	22	125	3.6	25.1
			42	2.1	7.1
			42*	2.1	13.4
			14	0.2	2.8
29	18	30	125	27.3	77.2
			42	1.7	7.7
			14	0.5	1.5
35	24	22	125	7.7	11.8
			125*	7.5	10.6
			42	3.5	6.9
			14	2.4	0.8
36	25	30	125	16.7	47.2
			42	1.6	2.2
			14	1.1	0.3

* In these treatments the air was not stirred during 5-day treatment period.

tubers could take up these very large amounts resulted only in a small quantity being absorbed and no differences were evident in the different treatments, although the amount of chlorhydrin present varied 8-fold. Here the permeability of the tubers to the chlorhydrin was clearly the

limiting factor and the extra chlorhydrin did no harm although under the same conditions sooner after harvest it would have most certainly resulted in producing injury.

Because of the wide range of effective concentrations reached in the tubers it is still possible to arrange treatments in such a way as to have the chlorhydrin absorbed sufficient to shorten the rest period in all stages of dormancy and low enough as not to cause any serious injury, as indicated in an earlier part of the paper. This is done by limiting the amount of chlorhydrin present to such an amount that even if it is practically all absorbed it is not enough to cause injury and if only partly taken up the concentration reached is still sufficient to effectively shorten the rest period. This, as a matter of fact, is what has been done in the previous experiments conducted over a number of years with many different lots of tubers in varying stages of dormancy. It is obvious that only a chemical with a wide range of effective concentrations could successfully be employed on a large scale under a variety of conditions as a vapor treatment. In the case of soak treatments in which the freshly cut surface is exposed evenly on all sides to a solution of definite concentration the quantity of chemical taken in by the tubers can be controlled much more accurately. If it is desired to treat whole tubers for subsequent shipping and later planting a soak treatment cannot be used; at least insofar as the experiments conducted in this laboratory have gone it has not been possible to introduce an effective concentration within the tubers when whole tubers were soaked in aqueous solutions of various chemicals.

Experiments with Peeled Tubers

The effect of the peel of the tubers in retarding the quantity of chlorhydrin taken in, as brought out in the data given above, can of course be directly demonstrated by comparing the chlorhydrin taken up by peeled and unpeeled tubers from the same lot. Even unpeeled tubers with just a small portion of the skin removed take up much more than similar uninjured tubers. Thus in some experiments with small tubers of the Irish Cobbler variety some two months after harvest in which the intact tubers took up only from 2 to 5 cc. of 0.1 molar chlorhydrin when exposed to 1 cc. of the 40 per cent solution for 18 hours, the tubers took up over twice as much under the same conditions if a small circle of skin 4 mm. in diameter was removed. As larger areas of skin were removed the absorption increased further so that when a portion 19 mm. in diameter was cut away the tubers took up 27 cc. of 0.1 molar chlorhydrin, or over half the chlorhydrin available. When similar tubers were peeled they took up practically all the chlorhydrin present and if more chlorhydrin were made available quantities up to 250 cc. 0.1 molar solution per tuber weighing about 40 grams were absorbed.

Effect of healing of the cut surface. The effect of healing of the cut surface is given by the data in Table VII. A number of tubers were peeled and tested at intervals after peeling for the quantity taken up both when exposed to the vapor and when soaked in an aqueous solution of chlorhydrin under standardized conditions. It is seen that there is a rapid drop of permeability as the suberization of the cut surface progresses. After four days the amount of chlorhydrin taken up when the tubers were

TABLE VII

EFFECT OF HEALING OF POTATO SURFACE ON QUANTITY OF ETHYLENE CHLORHYDRIN TAKEN UP FROM SOLUTION AND WHEN EXPOSED TO THE VAPOR

Days healed	Chlorhydrin absorbed			
	From solution*		In vapor treatment**	
	Cc. 0.1 M per 100 g.	Relative	Cc. 0.1 M per 100 g.	Relative
0	14.1 11.8	100	83.6 102.0	100
1	9.7 9.7	74	71.4 66.7	74
2	7.6 7.2	57	95.6 72.8	91
3	3.2 2.7	22	55.4 49.7	57
4	Trace	—	25.9 31.2	31
7	Trace	—	15.4 18.4	18
10	1.0† 0.7†		19.2 20.0	21
14	Trace†		10.7 9.6	11

* Tubers soaked for 0.5 hr. in solution containing 20 cc. 40 per cent chlorhydrin per liter.

** Individual tubers exposed for 2 hours to vapor from 1 cc. 40 per cent ethylene chlorhydrin in one liter container.

† Tubers soaked 4 hours.

soaked in an aqueous solution for 0.5 hour had dropped from 12.9 cc. 0.1 molar solution per 100 g. to a mere trace. When exposed to the vapor the tubers still took up appreciable quantities of chlorhydrin after 14 days of healing of the cut surface but this amounted to only 11 per cent of the 92.8 cc. 0.1 molar solution per 100 grams taken up immediately after cutting. These tests were carried out at 25.5° C.

Effect of temperature. As mentioned earlier in the paper, tests con-

ducted with whole tubers which were subjected to vapor treatments at different temperatures under otherwise similar conditions, showed that differences in temperature often seemed to have little influence on the quantity of chlorhydrin taken up. This was ascribed as being due to the limited permeability of the skin of the tubers which was not able to profit by the increased vapor pressure of the chlorhydrin at the higher temperatures. When the skin was removed increasing quantities of chlorhydrin were taken up with increased temperatures. The figures in Table VIII show that the value for Q_{10} is not far from 2.00 at the lower temperatures but becomes somewhat less above 15° C. In the case of soak treatments

TABLE VIII

EFFECT OF TEMPERATURE ON THE QUANTITY OF CHLORHYDRIN TAKEN UP BY PEELED TUBERS WHEN EXPOSED TO THE VAPOR AND WHEN SOAKED IN AQUEOUS SOLUTIONS

Temp. ° C.	Cc. 0.1 M chlorhydrin absorbed per 100 g. tubers	
	Vapor treatment*	Soak treatment**
1	29.9	—
5	43.6	24.4
10	61.3	25.2
15	83.0	32.7
20	99.4	27.0
25	109.7	32.3
30	134.7	33.0
35	132.4	32.3
53	126.7	40.6

* Single tubers were exposed to the vapor from 1 cc. of the 40 per cent solution in a one-liter container for 4 hours. Figures shown are averages of duplicate treatments at each temperature.

** Four tubers were soaked for one hour in 500 cc. of a solution containing 40 cc. of 40 per cent chlorhydrin per liter.

differences in temperature, as would be expected, have much less influence on the amount of chlorhydrin absorbed.

DISCUSSION

The fate of the ethylene chlorhydrin absorbed by the tubers during the treatment period is worthy of further study. It has been shown that the chlorhydrin slowly decomposes in the tissues (4). Preliminary tests have indicated that the decomposition in the tubers is more rapid than in buffers of similar pH values. It would be of interest to determine the products of this decomposition and their effect on dormancy. It is possible, of course, that the active agent in breaking the rest period may be a decomposition product of the chlorhydrin rather than the chlorhydrin itself.

When biological material, such as potato tubers, is subjected to the action of stimulative chemicals the effect produced depends upon the concentration. Below a certain minimum concentration the chemical will not have any measurable effect, then there will follow a range of concen-

tration in which the desired effect is secured and above this injury will result. The extent of this range from the minimum concentration having the desired effect to the maximum concentration which can be tolerated without injury is of great importance in the practical utilization of a chemical. This has long been recognized in human therapy in which the efficiency of a chemical is measured by the chemotherapeutic index which is the ratio between the maximum tolerated dose and the minimum curative dose (5, p. 2). For a chemical to be of practical value it is necessary that this index be high enough to permit its safe use. Similarly for a chemical to be of value for shortening the rest period it must have a proper margin of safety. The evidence presented in this paper adds to the evidence previously published showing that ethylene chlorhydrin can safely be used to break the rest period under a variety of conditions.

SUMMARY AND CONCLUSIONS

1. Dormant potato tubers were subjected to the vapor of ethylene chlorhydrin in closed containers for five-day periods under such conditions that the amount of chlorhydrin taken up by the tubers varied over a wide range. It was thus possible to determine the minimum concentration which was necessary to overcome the rest period and the maximum concentration which could be tolerated without serious injury.

2. On the basis of the quantities recovered from the tubers after treatment the effective range was found to be from 15 cc. to less than 1 cc. 0.1 molar chlorhydrin per 100 grams of tubers at 30° C. and from 25 cc. to less than 1 cc. 0.1 molar solution at 25° and 22° C. Some chlorhydrin is also decomposed within the tubers during the five-day treatment so that the actual amount taken in probably amounts to several cc. 0.1 molar more than the quantity recovered unaltered at the end of treatment.

3. It is not possible to control with any great degree of accuracy the quantity of chlorhydrin taken up by the tubers as a result of exposure to the vapor because the permeability of the skin varies greatly with time after harvest. Immediately after harvest the tubers are capable of taking up very large amounts of chlorhydrin but then the permeability drops very rapidly for the first week or ten days, after which the further decrease resulting is less rapid.

4. Because of the wide range of effective concentrations it is possible to regulate the amount of chlorhydrin available for absorption per unit of tubers in such a way that even if practically all the chlorhydrin were taken up, as in the case of tubers treated very soon after harvest, or if only half or less of the chlorhydrin were absorbed, the quantity taken in would still fall within this effective range.

5. The results reported herein were obtained in treatments in which only relatively small quantities of potatoes were treated at one time.

The exact conditions to use when large quantities are to be treated (quantities ranging from a bushel to a carload or more) can be determined only by actual experiment, but it is believed that enough information has been made available as a result of the experiments in this and earlier papers so that such treatments can be undertaken with a minimum of risk. It is suggested that the closed containers in which the treatment are made be not more than half full of tubers, that the treatments be continued for several days and the tubers held over for four days or more after the end of treatment before planting, and that the quantity of chlorhydrin used be adjusted to the quantity of the potatoes to be treated, using the effective range found above as a basis for estimating the chlorhydrin to be made available for absorption.

LITERATURE CITED

1. DENNY, F. E. Hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 118–125. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 59–66. 1926.)
2. ——— Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 386–396. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 169–180. 1926.)
3. ——— The importance of temperature in the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **15**: 395–404. 1928. (*Also in* Contrib. Boyce Thompson Inst. **1**: 373–382. 1928.)
4. DENNY, F. E., and LAWRENCE P. MILLER. Further experiments on shortening the rest period of potato tubers. Contrib. Boyce Thompson Inst. **7**: 157–181. 1935.
5. FINDLAY, G. M. Recent advances in chemotherapy. 532 pp. P. Blakiston's Son & Co., Inc., Philadelphia. 1930.
6. SIMON, JAR. Der Einfluss einiger Reizstoffe auf die Keimung und die Verkürzung der Periode der Wachstumruhe bei den Saatkartoffeln. Reprint Mitt. Tschechoslowak Akad. Landw. **6**(2). 4 pp. 1930.
7. STUART, WILLIAM, and E. H. MILSTEAD. Shortening the rest period of the potato. U. S. Dept. Agric. Tech. Bull. 415. 31 pp. 1934.

STORAGE TEMPERATURES FOR SHORTENING THE REST PERIOD OF GLADIOLUS CORMS

F. E. DENNY

INTRODUCTION

The results of the experiments of Loomis and Evans (5), Loomis (4), Emsweller (2), Fairburn (3), Volz and Keyes (7), and Volz (6) agree in showing the favorable effect of relatively high storage temperatures (30° to 35° C.) for the corms of gladiolus upon the rate of the development of the plant when the stored corms were subsequently planted.

However, these responses need not be interpreted as showing the superiority of high temperature storage for breaking dormancy, since it seems probable from the results of the present experiments that gladiolus corms which respond to high temperatures in this way are those that are not very dormant, or, at least, those which have passed partly through their rest period at the time they are exposed to the high temperature.

In a previous report (1) of experiments with small-sized corms (sizes Nos. 4 to 6, wt. 1 to 6 g. per corm), it was shown that, in the early stages of the rest period, storage at 3° C. or 10° C. was more favorable for hastening germination than at 29° or 35° C. A forcing effect at 35° C. was found only when the corms had reached the later stages of the rest period at the time the storage period was started.

In the present experiments similar tests have been carried out with the large-sized corms (Nos. 1 and 2) of several varieties, and the results show that for freshly-harvested corms storage at low temperatures (3° and 10° C.) favored early germination, high temperatures (30° and 35° C.) being ineffective at this stage of the dormant period. Later in the rest period, the higher temperatures caused earlier germination with certain varieties, other varieties, however, not responding to high temperature storage even at this period after harvest.

The experiments emphasize the effectiveness not of high but of low temperatures in shortening the rest period of gladiolus corms, and show the importance of the stage of dormancy of the corms in tests regarding the influence of storage temperature on the time required for germination.

METHODS

The corms, which were harvested October 5, 1935, were spread in a thin layer and allowed to dry for ten days at which time they were divided into two lots, samples from one being placed at once at the various storage temperatures, while the other lot was allowed to remain in burlap bags at room temperature for an additional period of 42 days before samples were taken for the storage periods at the various temperatures. Thus, the corms

TABLE I

EFFECT OF STORAGE TEMPERATURE UPON GERMINATION OF GLADIOLUS CORMS

Variety	Temp. of storage, ° C.	Storage started 10 days after harvest				Storage started 52 days after harvest			
		Stored 3 weeks		Stored 6 weeks		Stored 3 weeks		Stored 6 weeks	
		Days for 50% germ.	% Germ. after 60 days	Days for 50% germ.	% Germ. after 60 days	Days for 50% germ.	% Germ. after 60 days	Days for 50% germ.	% Germ. after 60 days
Souvenir	3	59	53	36	100	37	99	26	88
	10	58	59	33	100	40	100	30	100
	30	*	0	88	10	35	92	14	92
	35	90	18	78	25	25	100	14	92
Alice Tiplady	3	51	76	33	96	36	88	34	92
	10	59	64	34	100	40	100	31	94
	30	*	0	*	32	21	84	12	96
	35	86	0	31	66	18	96	13	96
Halley	3	*	48	27	88	24	100	22	100
	10	46	60	23	100	21	100	14	100
	30	*	0	*	0	*	0	*	30
	35	*	0	*	12	*	7	33	56
Mr. W. H. Phipps	3	44	84	30	100	22	100	14	97
	10	44	90	31	100	21	95	16	98
	Room	86	7	76	13	52	60	26	82
	30	*	0	*	7	58	52	14	94
	35	89	10	74	35	22	77	14	97
Giant Nymph	3	49	71	24	100	22	100	14	100
	10	54	59	23	100	20	100	14	97
	Room	*	0	*	3	54	70	32	95
	30	*	0	83	10	49	60	17	100
	35	*	0	*	33	38	95	14	97
Mrs. Peters	10	*	6	41	93	53	59	33	93
	30	*	0	*	0	*	15	74	34
Pendleton	10	75	15	44	94	46	100	28	100
	30	*	0	*	0	*	13	50	56
Bennett	10	45	81	23	100	29	88	22	93
	30	*	0	*	0	*	0	39	66

Note: Days counted from time of planting.
* 50% germination not reached within 90 days.

of the first lot were in a very dormant condition at the time the temperature tests were begun (i. e., at 10 days after harvest), while those of the second lot had passed at least partly through the rest period before exposure to the various temperatures (i. e., at 52 days after harvest).

The number of corms in each sample at the different temperatures varied from 18 to 35 according to the supply of corms available for each variety. The samples of corms were stored in paper bags in rooms the

temperatures of which were maintained thermostatically at the temperatures shown in Table I, except in the case of "room" temperature, which in the present tests was approximately 22° C. At the end of the storage period the corms were planted in moist soil in flats and kept at room temperature.

RESULTS

The effect of storage temperatures and of the duration of storage at each temperature upon germination is shown in Table I, columns 3 to 6 referring to corms placed in storage 10 days after harvest, and columns 7 to 10 referring to those not placed at the storage temperatures until 52 days after harvest. The reader will note essential differences in the response at these two stages of the rest period, especially by the varieties Alice Tiplady and Souvenir.

With the lots started at the 10-day period after harvest, 3° and 10° C. were much more effective than higher temperatures. A storage period of only 3 weeks at these low temperatures hastened germination of all varieties except Mrs. F. C. Peters and Mrs. Frank Pendleton, inducing 50 per cent germination within 44 to 59 days, while higher temperatures either required 86 to 90 days for 50 per cent germination, or failed to bring about germination to that extent. Storage at 30° C. was particularly ineffective since with no variety in the test was 50 per cent germination obtained within 90 days from time of planting.

The varieties Mrs. F. C. Peters and Mrs. Frank Pendleton responded to six weeks' storage at 10° (see Table I, columns 5 and 6), reaching the 50 per cent stage in 41 to 44 days after planting, and showing more than 90 per cent germination after 60 days, whereas after storage for 6 weeks at 30° C. no sprouts were obtained after 60 days. After six weeks' storage starting at the 10-day period after harvest all varieties responded better to low than to high temperatures, except Alice Tiplady, in which case 35° C. produced a gain of 2 to 3 days over 3° or 10° C., even though the per cent germination was not as high. A storage temperature of 30° for six weeks, however, was ineffective at this stage even with Alice Tiplady. The gain in the time to reach 50 per cent germination by storage for six weeks at 10° as compared with 30°, was 55 days for Souvenir, and 60 days for Giant Nymph, but the gains cannot be computed for the other varieties tested, since the 50 per cent stage was not reached with any of them at the 30° temperature.

When the storage at the different temperatures was not begun until 52 days after harvest (see Table I, columns 7 to 10) the results varied with the different varieties. The two low temperatures, 3° and 10° C., produced earlier germination with Halley, Mrs. F. C. Peters, Mrs. Frank Pendleton, and Dr. F. E. Bennett, whether the storage period was for three or six

weeks. The two high temperatures, 30° and 35° C. gave earlier germination with Alice Tiplady and Souvenir, and also were at least equal to 3° or 10° with Mr. W. H. Phipps and Giant Nymph, especially if the storage period was as long as six weeks. With the two varieties with which a test was made at room temperature, Mr. W. H. Phipps and Giant Nymph, this temperature was less favorable than temperatures either above or below it.

SUMMARY

At two different stages of the rest period, i. e. at 10 days and at 52 days after harvest, gladiolus corms of eight different varieties were stored for three weeks and for six weeks at various temperatures and were planted.

When the storage period was started at the 10-day stage after harvest, temperatures of 3° and 10° C. gave much earlier germination than temperatures of 30° and 35° C. with all of the varieties that were tested. Even three weeks' storage at the low temperatures hastened germination of all varieties except Mrs. F. C. Peters and Mrs. Frank Pendleton, and these two responded to six weeks' storage at these temperatures. Storage at this stage at 30° was particularly ineffective, 50 per cent germination not being obtained within 90 days after planting.

When the corms were not placed under the experimental storage conditions until 52 days after harvest, the temperature effect varied with the variety tested; low temperature storage gave earlier germinations with the varieties Halley, Mrs. F. C. Peters, Mrs. Frank Pendleton, and Dr. F. E. Bennett; high temperature storage gave earlier germinations with Alice Tiplady and Souvenir; with the varieties Mr. W. H. Phipps and Giant Nymph six weeks' storage at this stage of dormancy showed no differences between 3° and 35° C.

LITERATURE CITED

1. DENNY, F. E., and LAWRENCE P. MILLER. Storage temperatures and chemical treatments for shortening the rest period of small corms and cormels of gladiolus. Contrib. Boyce Thompson Inst. **7**: 257–265. 1935.
2. EMSWELLER, S. L. Some results of storing gladioli at various temperatures. Proc. Amer. Soc. Hort. Sci. **27**: 550–553. 1931.
3. FAIRBURN, DAVID C. Growth responses of the gladiolus as influenced by storage temperatures. Iowa Agric. Exp. Sta. Res. Bull. 170: 93–123. 1934.
4. LOOMIS, W. E. Forcing gladiolus. Proc. Amer. Soc. Hort. Sci. **30**: 585–588. 1934.
5. LOOMIS, W. E., and M. M. EVANS. Experiments in breaking the rest period of corms and bulbs. Proc. Amer. Soc. Hort. Sci. **25**: 73–79. 1929.
6. VOLZ, E. C. Storage of gladiolus corms. Iowa Agric. Exp. Sta. Rept. Agric. Res. 1933–34: 152. 1934.
6. VOLZ, E. C., and C. G. KEYES. Gladiolus forcing: effects of exposure to high temperatures before planting. Proc. Amer. Soc. Hort. Sci. **30**: 583–584. 1934.

LONGEVITY OF POLLEN OF LILIUM AND HYBRID AMARYLLIS

NORMA E. PFEIFFER

As a result of interest in the longevity of pollen, a number of short papers have appeared, most of which have been considered in the reports of Pfundt (17), Knowlton (11), and Holman and Brubaker (6). In general, the effect of light, humidity, temperature, and reduced pressure have been considered singly or in various combinations, on one or more species, as convenience or purpose dictated. In the lists only scattered data were available for the genus *Lilium*, so that the best conditions for storing the pollen for future use were not known. To find a better method than the usual storage over calcium chloride at room temperature, a study of the effect of temperature, humidity, and reduced pressure on the retention of viability of pollen in four species of *Lilium* was undertaken. This was extended to include pollen of another interesting horticultural form *Hippeastrum vittatum* Herb. (hybrid *Amaryllis*).

The species used were *Lilium auratum* Lindl., *L. longiflorum* Thunb., *L. philippinense* Baker, and *L. speciosum* Thunb. Of these the only previous definite report is that of Horsford (7) who saved pollen of late blooming *L. auratum* wrapped in several sheets of paraffin paper until the following spring when it was successfully used on *L. martagon* to produce one capsule. Horsford further reported that most of the pollen of early sorts (not specified) saved in small envelopes, was good for two or three months. For other species there are the following records of longevity: by Mangin (13) 14 days for *L. candidum* and *L. croceum* when air-dry; by Molisch (14) 67 days for *L. candidum* and *L. bulbiferum*, air-dry; and by Pfundt (17) 41 days for *L. bulbiferum*, air-dry, and 142 days either at 30 per cent humidity or over concentrated sulphuric acid. For the family *Liliaceae* the mean longevity of pollen of the forms previously investigated (6, p. 198) is 23 days in the air-dry condition and 86 days when stored at the most favorable humidity.

METHODS

Stamens were collected on the day of the opening of the flower before the release of pollen and allowed to stand in clean dishes until the pollen was shed. Lily pollen was collected in August, *Amaryllis* in November and later. Due to its oily character the pollen of *Lilium* was easily rolled into little masses separable from the stamen tissue. Usually this was done; occasionally the stamen with its adhering pollen was stored. No apparent difference in results was observed. In some specified cases the pollen was allowed to stand in the laboratory or kept in a calcium chloride desiccator for two days before storage.

Previous tests (15) have shown that darkness is slightly more favorable than light, so that all storage was in darkness. The simplest condition was storage of small amounts of pollen in gelatin capsules at temperatures of 1°, −5°, and −17° C.; for *Lilium* the last two temperatures were maintained about 70 days when it was necessary to move the material to a room where the temperature fluctuated for several days, attaining almost zero at times, but was finally brought to −11° C. For *Amaryllis*, −11° C. was the temperature used throughout the experiment. In the capsules, the humidity was not controlled; in the cold rooms the air was near saturation for the temperature.

Two series were run in desiccators with humidity controlled by means of anhydrous calcium chloride, concentrated sulphuric acid, and definite concentrations of the acid. The humidities represented were 1.2 per cent (sulphuric acid), 1.4 per cent (calcium chloride), and approximately 35, 50, 65, and 75 per cent of saturation (49, 43, 36, and 30 per cent sulphuric acid). One set of desiccators was held at room temperature, the other at 10° C. No correction for temperature difference was made in the solutions, so that there is a slight discrepancy between the two series as to actual humidity.

The vacuum set was arranged by sealing off five-inch lengths of glass tubing at one end. A sample of *Lilium* pollen was put into each, the tube exhausted by means of a vacuum pump, and sealed off at the open end. While still hot, this end was covered with de Khotinsky cement to provide for slower cooling and avoid cracking of the glass. In these tubes there was no necessity of using a cotton plug as employed by Kellerman (10) because of the heavy oily character of the pollen which massed in a globule at the bottom of the tube. The evacuated tubes were stored at 20°, 5°, and −5° C.

At intervals samples were taken from each of the lots and tested for germination by sowing them in a hanging drop of a suitable solution in a van Tieghem cell with water in the base of the cell. After testing a number of media based on the methods of other investigators (1, 5, 8, 9, 12, 13, 14, 16) a solution of 7 per cent cane sugar, 1.5 per cent gelatin, with the addition of yeast as recommended by Brink (2), was used for *Lilium* species, and 7 per cent cane sugar, 1 per cent agar, and yeast for *Amaryllis*. An effort at even distribution of grains was made both for the effect upon each other (3) and for ease in counting. The tests were run in duplicate; counts were made of pollen grains forming apparently functional tubes as compared with the total number of grains, in a representative number of fields. Usually two to three hundred grains were counted, sometimes as many as five to eight hundred. Since the pollen is rather large in size (*Lilium* about 120μ and *Amaryllis* about 95μ), relatively low magnifications of about 30 to 50 diameters were used in making the counts.

POLLEN OF LILIUM SPECIES

The best results were obtained with storage at temperatures lower than room temperature in this order: at 10° C. with intermediate humidities of 35, 50, or 65 per cent, at −5° C. in capsules with no control of the humidity, at −5° C. with reduced pressure, and at −17° C. in capsules. At 10° C., some pollen in all four species of *Lilium* was capable of producing tubes in the intermediate humidities after seven months' storage. In the capsules at −5° and −17° C., and under reduced pressure at −5° C., there were fewer tubes in some species, or none in the least resistant forms, at the end of the same interval.

ATMOSPHERIC PRESSURE

Storage at 10° C. The percentages of germination at this temperature with relative humidities of 50 and 65 per cent are high during the first few months of storage, as shown by results of duplicate tests in Table I, columns 4 and 5, with tubes formed by 40 to 60 per cent of the pollen grains in most cases. Later, differences between the lots show more sharply. At the end of seven months (column 10), at 50 per cent humidity, *L. auratum* gives 22 and 26 per cent germination, *L. longiflorum* 34 and 24 per cent, *L. philippinense* (air-dry) 8 and 7 per cent, *L. speciosum* 32 and 13 per cent germination. At 65 per cent humidity, the percentages of germination are: *auratum* no test, *longiflorum* 63 and 46, *philippinense* 12 and 11, and *speciosum* 10 and 8 per cent. At 35 per cent humidity (not tabulated) the percentages of germination are in the same general range for three of the four species; *auratum* 44 and 46, *longiflorum* 15 and 13, *philippinense* 10 and 4, and *speciosum* 16 and 12 per cent. Apparently *auratum* and *longiflorum* are the most resistant of the four pollens, with *auratum* surviving somewhat better at 35 per cent humidity, and *longiflorum* better at 65 per cent. *Speciosum* pollen retains viability best at 50 per cent humidity. The *philippinense* lot used is not exactly comparable, since it was exposed to drying in the air before storage, while the others shown in the table were fresh. But a comparison with *auratum* pollen previously air-dried indicates that this survives better than *philippinense*, with percentages in storage at 35, 50, and 65 per cent humidity of 24 and 14, 11 and 16, 17 and 14 for *auratum*, and 10 and 4, 8 and 7, and 12 and 10 for *philippinense*, after seven months.

At 75 per cent humidity, *auratum* and *longiflorum* retain viability (64 and 53 per cent germination) for two months, when the other two species give low results (4 to 10 per cent). After three months, all germinate poorly.

The very low humidity over concentrated sulphuric acid or calcium chloride is not favorable as a storage condition. Most of the pollen of all species loses its viability within a month.

TABLE I

EFFECT OF STORAGE TEMPERATURE AND HUMIDITY ON VIABILITY OF LILIUM POLLEN

Species	Relative humidity %	Temp., ° C.	Per cent germination						
			Months of storage						
			1	2	3	4	5	6	7
Auratum	50	10	76	50	37	39	72	33	22
			77	59	28	48	58	39	26
	65	10	75	52	64	69	32	15	—
			90	55	34	55	36	21	—
	—*	−5, −11**	70	68	41	50	33	36	31
			75	61	57	56	30	32	32
	—	−17, −11**	67	46	17	20	—	15	10
			73	50	18	18	—	15	13
Longiflorum	50	10	63	41	25	12	19	30	34
			70	43	32	17	19	32	24
	65	10	70	48	65	49	46	52	63
			74	38	53	50	52	50	46
	—	−5, −11**	67	60	42	46	12	11	5
			81	66	53	41	11	9	4
	—	−17, −11**	59	47	9	6	—	2	2
			56	47	7	2	—	2	1
Philippinense (air-dry)	50	10	49	23	11	17	13	13	8
			31	20	12	8	17	19	7
	65	10	53	13	21	13	22	14	12
			53	15	21	7	25	14	11
	—	−5, −11**	42	40	19	13	6	7	4
			46	41	18	12	8	6	4
	—	−17, −11**	43	20	2	1	—	0	0
			55	23	6	0	—	0	0
Speciosum	50	10	25	14	—	—	34	24	32
			40	15	—	—	31	22	13
	65	10	41	29	20	13	15	18	10
			42	20	12	10	17	13	8
	—	−5, −11**	42	26	10	1	1	2	1
			58	23	16	1	1	1	1
	—	−17, −11**	60	25	1	0	—	1	0
			55	27	0	1	—	1	0

* Dash indicates pollen in gelatin capsules with no control of humidity; at 50 and 65 per cent relative humidity in desiccators over concentrations of sulphuric acid.

** For two months at first temperature, subsequently at −11° C.

Storage at $-5°$ *C. Auratum* and *longiflorum* pollen hold up well for four months when stored in gelatin capsules without humidity control at $-5°$ C. for two months, then at $-11°$, with germination of 50 and 56 per cent and 46 and 41 per cent (Table I, column 7). About 40 per cent of *philippinense* pollen and 25 per cent of *speciosum* pollen remain viable after two months with a rapid drop in germinating power thereafter. At the end of seven months (Table I, column 10) only *auratum* shows fair retention of viability, 31 and 32 per cent.

Storage at $-17°$ *C.* Most of the pollen retains viability in lesser degree where stored in gelatin capsules for two months at $-17°$ than at $-5°$ C. (Table I, column 5), *auratum* 46 and 50 per cent, *longiflorum* 47 and 47, *philippinense* 20 and 23, and *speciosum* 25 and 27. After removal to $-11°$ C., which was accompanied by an unavoidable fluctuation in temperature, germinating ability dropped sharply in the subsequent month, with *auratum* at 17 per cent and the others below 10 per cent.

Storage at $1°$ *C.* This temperature used for storage of pollen in gelatin capsules, with no humidity control, gave good results in two months for *auratum* with 49 and 43 per cent germination, and for *longiflorum* with 40 and 43 per cent, but only an occasional tube in the other species. In three months, germination drops to 24 and 25 per cent for *auratum* and 16 and 27 per cent for *longiflorum*. Thereafter the percentage decreases until no tubes are produced in any species at the end of five months.

Storage at room temperature. Pollen stored in the humidities tried failed to live as long at room temperature as that at 10° C. In 50 and 65 per cent humidity, germination percentages are apt to be lower here after two months than in 10° after seven months. A single set of experiments in humidities of 10 and 25 per cent, tested after four months' storage showed more germination than in either higher or lower humidities; in *auratum* and *longiflorum*, 30 per cent or less germinated.

REDUCED PRESSURE

Storage at $-5°$ *C.* As in storage under atmospheric pressure at this temperature, the percentages of germination are satisfactory for three of the species even after seven months under reduced pressure. The results are as follows: *auratum* 38 and 39 per cent, *longiflorum* 16 and 20, and *philippinense* 10 and 11. The method is not advantageous for *speciosum* pollen which fails to produce tubes after four months.

Storage at $5°$ *C.* This is an unsatisfactory method of storage, although about 13 per cent of the pollen grains of both *auratum* and *longiflorum* germinate after storage for five months. Results were less consistent both in pairs of tests and in tests made on pollen taken from different evacuated tubes, in spite of use of the same precautions as in sowing pollen grains

in other series. Inconsistency may be due to difference in germinating power of grains inside the pollen mass and those on the surface.

Storage at 20° C. Pollen stored at this temperature dies even sooner than at 5° C.; only a low percentage of grains form tubes after two months, with the exception of *longiflorum*, in which 16 and 21 per cent germinate after seven months.

POLLEN OF HYBRID AMARYLLIS

Amaryllis pollen taken from red, white, and striped flowers of *Amaryllis*[1] germinated well after storage in humidities of 35, 50, and 65 per cent at 10° C. Almost equally good results followed storage in gelatin capsules at −11° C. without humidity control. Pollen stored in more moist or drier air at 10° or in the intermediate humidities at room temperature, retained its vitality for shorter intervals. Pollen was not stored here under reduced pressure.

HUMIDITY CONTROLLED

Storage at 10° C. Pollen held at 35 and 50 per cent relative humidity for five months at this temperature germinated to the extent of averages of 51 to 80 per cent for duplicate tests in the different lots (Table II). Pollen lives almost as well for the first four months in a humidity of 65 per cent but falls off greatly in germinating ability except in the red, 41 and 46 per cent at five months. At 75 per cent humidity, pollen from red flowers produces tubes in 50 and 41 per cent of the grains, from striped flowers in 16 and 23 per cent, from white only an occasional tube, after two months. At three months, there is very little germination, even in the red.

Amaryllis pollen survived in the drier air over sulphuric acid and calcium chloride better than the *Lilium* lots. After storage over calcium chloride for two months, percentages of germination are red 50 and 30, white 21 and 12; after four months, red 23 and 20, white and striped only 4 to 7 per cent. Pollen over sulphuric acid retains viability to some extent for two months, red 16 and 12, striped 11 and 10, white 11 and 8; the following month there is even less tube production, and in four months, none whatever.

Storage at room temperature. Retention of viability at this temperature was best brought about by storage in 35 per cent humidity, with good results after two months (red 73 and 68 per cent, striped 53 and 58, white 56 and 62 per cent). A portion of the pollen grains germinate after four months' storage, red 30 and 48 per cent, striped 16, white 7 and 12, but only sporadic cases of tube formation occur thereafter. At 50 per cent humidity, germination is only slightly less after two months, red 71 and

[1] Experimental plants from bulbs supplied by I. W. Heaton, Orlando, Florida.

67, striped 55 and 65, white 33 and 34; relatively few pollen grains remain viable after four months and none after five.

Both higher and lower humidities fail to prolong life for two months. Only an occasional grain germinates after storage over concentrated sulphuric acid or calcium chloride, and none in 65 or 75 per cent humidity.

HUMIDITY NOT CONTROLLED

Storage at $-11°$ *C.* Tests made with one lot of pollen from red flowers and two from striped showed great viability over a period of five months

TABLE II

EFFECT OF STORAGE, TEMPERATURE, AND HUMIDITY ON VIABILITY OF AMARYLLIS POLLEN

Relative humidity %	Temperature	Per cent germination											
		Flowers red				Flowers striped				Flowers white			
		Months of storage				Months of storage				Months of storage			
		2	3	4	5	2	3	4	5	2	3	4	5
35	10° C.	58	56	54	64	26	48	63	62	49	46	44	49
		54	52	62	58	11	22	68	62	43	48	50	55
	Room	73	34	48	2	53	28	16	0	62	21	7	0
		68	29	30	1	58	11	16	0	56	23	12	0
50	10° C.	72	68	67	76	31	33	67	68	58	43	70	58
		70	69	74	84	38	21	50	66	48	45	67	62
	Room	71	45	22	0	55	12	4	0	34	11	1	0
		67	30	20	0	65	15	4	0	33	9	1	0
65*	10° C.	82	62	65	41	38	61	31	5	51	32	27	7
		83	60	73	46	37	66	55	5	51	42	40	6
—**	−11° C.	86	73	57	62	75†	55†	55†	49†	—	—	—	—
		83	67	59	72	65†	50†	39†	45†	—	—	—	—

* In room temperature at 65 per cent humidity, there was no germination.
** In gelatin capsules, no control of humidity.
† Average of two sets.

(Table II). As in the series with controlled humidity the best results were obtained with that from red flowers, 72 and 62 per cent as compared with 50 and 54 and 40 and 44 for the two others. Pollen from white flowers, which in other tests showed slightly lower germination percentages, was not available for this test.

DISCUSSION

The results agree with the conclusions reached by Pfundt (17) that there is a close relation between pollen longevity and moisture content of the air, with maxima and minima of different pollens in different humidities. Pfundt found fewer pollens surviving well at 60 or 90 per cent

humidity than at 30 per cent or even over sulphuric acid, with very few equally good over a wide range in moisture content of the air. Holman and Brubaker (6) found almost the same humidities as Pfundt the most effective for conserving vitality of most of the pollens they tested.

Clearly in the experiments with *Lilium* and *Amaryllis* there are individual differences between the pollens used. But in both genera, pollen survives poorly in the dry atmospheres and better in intermediate humidities, even up to 65 per cent, especially at 10° C. There is some indication that at room temperature, a lower humidity serves to increase the length of life, as in *Amaryllis* where the maximum at room temperature occurs in 35 per cent humidity as compared with the maximum at 10° in 50 per cent humidity. But for neither genus does this extend to the very dry air over calcium chloride or sulphuric acid. Roemer (18) experimented with pollen stored at room temperature and 5° to 10° C. with and without desiccators. Most of the seven dicotyledonous forms used gave good germination after storage in desiccators at 5° to 10° C. for 45 days, poorer germination after two months, and only occasional or no tubes after three months. His results for the same temperature without desiccator, and for room temperature with desiccator, were usually lower. This was tested only in part in our experiments, since the set lacking humidity control was at a lower temperature; the good results in capsules at −5° or −11° C. were probably partly due to the oily character of the pollen which served to protect it against drying out. This is similar to results of Knowlton (11) on *Antirrhinum* pollen which remains viable longest at a low temperature (0° to −17° C.). Goff (4) had earlier found that pollen of fruit trees remained viable after short exposure to low temperatures, a point since substantiated by other workers.

In the experiments on *Lilium* at reduced pressure, storage at best appears no better than at atmospheric pressure with comparable temperatures, as seen in the −5° C. series. Results are less consistent, however, at reduced pressure, so that it appears less dependable. Knowlton (11) has previously found that *Antirrhinum* pollen remained viable a shorter time with reduced pressure either at 10° or 0° C. Kellerman (10) on the other hand reported it as a favorable method for storing citrus pollen.

Data for retention of viability at the most favorable humidity have been summarized (6, p. 198) for ten families, among which the *Liliaceae* rank fifth with 86 days as mean longevity of pollen, and *Amaryllidaceae* sixth with 75 days. In the present work, pollens of the two genera have not been stored equally long, but it is apparent that there is retention of viability for even longer intervals (more than 210 days in *Lilium* and 150 days in *Amaryllis*) with favorable temperatures.

SUMMARY

Two methods are especially recommended for the conserving of viability in four species of *Lilium* and in hybrid *Amaryllis:*

1. Storage in relative humidities of 35, 50, and 65 per cent at 10° C. in which *Amaryllis* retained viability more than five months and *Lilium* more than seven months. There are specific differences as to the most favorable humidity in this range, with 35 per cent best for *auratum*, 50 for *speciosum*, and 65 for *longiflorum*.

2. Storage in gelatin capsules with no humidity control, at −5° C. for *Lilium* and −11° for *Amaryllis*.

Almost equally favorable results are obtained under reduced pressure at −5° C. for *Lilium*, but with the expenditure of more labor.

Less favorable methods are listed among the following:

1. Storage in capsules at −17° C. is less effective than −5° C., and 1° less so than −17°.

2. Storage at room temperature with controlled humidity does not conserve viability as long as at 10° C. In the most advantageous humidities, germination in *Lilium* is about as good after two months at room temperature as after seven months at 10° C.; viability is retained somewhat better by *Amaryllis* than by *Lilium*.

3. The very dry air over sulphuric acid or calcium chloride and the high relative humidity of 75 per cent, are less favorable for retention of viability either at 10° C. or room temperature than the intermediate relative humidities of 35, 50, and 65 per cent. *Amaryllis* gives better germination in the lower range of humidity than is obtained with *Lilium*.

LITERATURE CITED

1. BRINK, R. A. The physiology of pollen. I. The requirements for growth. Amer. Jour. Bot. **11**: 218–228. 1924.
2. ——— The physiology of pollen. II. Further considerations regarding the requirements for growth. Amer. Jour. Bot. **11**: 283–294. 1924.
3. ——— The physiology of pollen. IV. Chemotropism; effects on growth of grouping grains; formation and function of callose plugs; summary and conclusions. Amer. Jour. Bot. **11**: 417–436. 1924.
4. GOFF, E. S. A study of certain conditions affecting the setting of fruits. Wisconsin Agric. Exp. Sta. Rept. **18**: 289–303. 1901.
5. GREEN, J. REYNOLDS. Researches on the germination of the pollen grain and the nutrition of the pollen tube. Phil. Trans. Roy. Soc. London B. **185**: 385–409. 1894.
6. HOLMAN, RICHARD M., and FLORENCE BRUBAKER. On the longevity of pollen. Univ. California Publ. Bot. **13**: 179–204. 1926.
7. HORSFORD, F. H. Longevity in lily pollen. Jour. Heredity **9**: 90. 1918.
8. HUREL-PY. Recherches sur les conditions du pH nécessaires pour obtenir la germination des grains de pollen et la coloration vitale de leurs vacuoles. Compt. Rend. Acad. Sci. [Paris] **198**: 195–197. 1934.
9. JOST, L. Über die Selbststerilität einiger Blüten. Bot. Zeit. **65**: 77–118. 1907.

10. KELLERMAN, MAUDE. Successful long-distance shipment of citrus pollen. Science **42**: 375–377. 1915.
11. KNOWLTON, H. E. Studies in pollen, with special reference to longevity. New York [Cornell] Agric. Exp. Sta. Mem. 52: 751–793. 1922.
12. LIDFORSS, BENGT. Weitere Beiträge zur Biologie des Pollens. Jahrb. Wiss. Bot. **33**: 232–312. 1899.
13. MANGIN, LOUIS. Recherches sur le pollen. Bull. Soc. Bot. France **33**: 337–342; 512–517. 1886.
14. MOLISCH, HANS. Zur Physiologie des Pollens, mit besonderer Rücksicht auf die chemotropischen Bewegungen der Pollenschläuche. Sitzungsber. Akad. Wiss. Wien. Math. Naturwiss. Kl. Abt. 1. **102**: 423–449. 1893.
15. NOHARA, SIGEROKU. Experimental studies on pollen of some *Salix*. Jap. Jour. Bot. **2**: 1–34. 1924.
16. PATON, JULIA BAYLES. Pollen and pollen enzymes. Amer. Jour. Bot. **8**: 471–501. 1921.
17. PFUNDT, MAX. Der Einfluss der Luftfeuchtigkeit auf die Lebensdauer des Blütenstaubes Jahrb. Wiss. Bot. **47**: 1–40. 1909.
18. ROEMER, THEODOR. Zur Pollensaufbewahrung. Zeitschr. Pflanzenzücht. **2**: 83–86. 1914.

THE ACTION OF FUNGOUS SPORES ON BORDEAUX MIXTURE[1]

S. E. A. McCallan and Frank Wilcoxon

When a solution of copper sulphate is treated with slaked lime as in the preparation of Bordeaux mixture, a reaction takes place by which the copper is precipitated in a form which has an exceedingly low solubility in water. Since, however, this precipitate is extremely toxic to many species of fungous spores, there have been numerous investigations to determine the means by which the relatively insoluble copper in the Bordeaux precipitate becomes transformed, in part at least, to soluble copper. The three principal hypotheses have been stated by Barker and Gimingham (1, p. 76) as follows: "1. That copper is brought into solution by atmospheric agencies, more especially by the action of the carbon dioxide of the air, i.e., a purely chemical explanation. 2. That the leaves on to which the mixture is sprayed, exert a solvent action on the compounds, i.e., an action of the host plant. 3. That the fungus itself is responsible for the production of the soluble copper by which it is finally poisoned, i.e., an action of the fungus."

It has been pointed out previously (7) that the second hypothesis is inadequate to explain entirely the fungicidal action, since Bordeaux mixture is toxic when sprayed on glass slides, where a possible solvent action of the leaf excretions cannot be a factor. The evidence regarding the first hypothesis, namely that copper is brought into solution by atmospheric agencies, is contradictory, and many of the experiments in support of it have been performed with fresh Bordeaux precipitate, or using excessive amounts of carbon dioxide, and hence the conditions were different from those under which Bordeaux normally exerts its toxic action.

The third hypothesis, that the spore itself is responsible for the production of soluble copper has also been the subject of a number of investigations. The most recent of these (7) gives an extensive summary of the previous work on the fungicidal action of Bordeaux mixture. In this article it was shown that when a collodion sac containing Bordeaux mixture was suspended in distilled water containing fungous spores, the water acquired the property of being toxic to fungous spores in subsequent tests. If the collodion sacs were suspended in distilled water without spores, the water did not acquire toxic properties in subsequent spore germination tests. Furthermore, when spores were allowed to germinate in distilled water, and this water after filtering off the spores was placed over dried Bordeaux mixture, it became toxic.

In these previous experiments chemical methods of detecting and

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 114.

estimating copper were not used, and quantitative studies of the amount of copper in solution were not made. Recently several new and extremely sensitive chemical tests for copper have been devised (3, 12). In the present paper a further study has been made of the action of spores on Bordeaux mixture, making use of these more sensitive tests.

MATERIALS AND METHODS

The fungous spores employed in this study were conidia of *Sclerotinia fructicola* (Wint.) Rhem. (16), *Botrytis paeoniae* Oud., *Alternaria solani* (Ell. & Mart.) Jones & Grout[2] (17), *Glomerella cingulata* (St.) Sp. & von S.,[2] *Neurospora sitophila* (Mont.) Shear & Dodge, *Aspergillus* sp., *niger* group (13), and uredospores of *Uromyces caryophyllinus* (Schr.) Wint.

The uredospores of *U. caryophyllinus* were obtained from naturally infected greenhouse carnations. All of the other fungi were cultured on potato dextrose agar at room temperature. The spores obtained from *Sclerotinia fructicola* were from 5 to 10-day-old cultures, while in the case of the other species the age was from 10 to 14 days.

The moist chamber method of determining toxicity was employed (6, 8, 9, 16). Spores of *S. fructicola*, *B. paeoniae*, *A. solani*, *G. cingulata*, and *U. caryophyllinus* were germinated both in distilled water and in water containing 0.02 per cent filtered orange juice (14). Since the spores of the obligate saprophytes *N. sitophila* and *A. niger* will not germinate in distilled water it is necessary to add 2 per cent filtered orange juice in order to obtain satisfactory germination.

For the estimation of copper in concentrations of from 1 to 10 parts per million, the method of Callan and Henderson (3) was employed, as described by Hockenyos (4). This method depends on the fact that the reagent sodium diethyl dithiocarbamate gives a yellowish color with very dilute copper solutions. The test is performed in ammoniacal solution, and the unknown and standard may be compared in a colorimeter.

Copper in concentrations below 1 p.p.m., as in the determination of the solubility of the film of dried Bordeaux mixture in distilled water, was estimated by a method devised by Quartaroli (12). This method depends on the fact that minute traces of copper catalyze the decomposition of hydrogen peroxide in ammoniacal solution. A 10 cc. sample of the unknown is treated with 1 cc. of 3 per cent hydrogen peroxide and 2 drops of concentrated ammonia, rapidly mixed and poured into a fermentation tube. Evolution of oxygen begins and continues at a rate depending on the concentration of copper.

[2] Cultures of *Alternaria solani* and *Glomerella cingulata* were kindly furnished by Dr. Robert O. Magie, New York Agricultural Experiment Station, Geneva, New York.

SOLUBILITY OF COPPER IN BORDEAUX PRECIPITATE

When copper sulphate and lime suspensions are mixed in such proportions that one part of lime is used to one part of copper sulphate, practically all the copper in solution is precipitated. Hockenyos (4) has published the results of the determination of copper in the supernatant liquid above such a precipitate, and has found 1.4 p.p.m. of copper. The present authors have investigated the solubility of 4–4–50 Bordeaux when the precipitate was filtered and washed ten times with distilled water. The precipitate was resuspended in distilled water and after one hour 1 p.p.m. of copper was found in solution. In making the test, the suspension was centrifuged, and the sample pipetted off in order to avoid possible adsorption of copper by filter paper. In making the determinations, the method used was that of Callan and Henderson (3) referred to previously. From these results it appears that appreciable amounts of copper remain in solution in the mother liquor of 4–4–50 Bordeaux mixture, and that the precipitate, after filtering and washing, gives up approximately the same amount of copper to distilled water in which it is suspended.

However, these results have little bearing on the mode of action of Bordeaux mixture as usually applied, since they were obtained with moist precipitates, while in actual practice the precipitate usually has an opportunity to dry out on the foliage and is subsequently moistened by rain or dew. When 4–4–50 Bordeaux was sprayed on the inside of moist chambers, 20 cm. in diameter, and the latter placed to dry in the sun for 2 to 4 hours, the solubility of the copper was found to be considerably less. After the chambers were dried 50 cc. of distilled water were placed in them, and they were allowed to stand overnight. The copper was determined after centrifuging by the method of Quartaroli (12). Under these conditions the solubility of the copper had fallen to a value of 0.2 to 0.3 p.p.m. This concentration of copper is insufficient to affect materially the germination of spores of the species of fungi used.

SOLUBILIZING ACTION OF SPORE FILTRATES

With the highly sensitive methods for detecting copper now available, it should be readily possible to determine by purely chemical means, whether or not spores or spore filtrates are capable of rendering the copper in dried Bordeaux mixture soluble. Accordingly, 50 cc. suspensions of spores in water were placed overnight in sprayed and dried moist chambers. The spores were obtained by washing test tube cultures of *Sclerotinia fructicola* and of *Botrytis paeoniae*, after which the spores were washed twice by centrifuging. Washings from four sterile tubes of potato dextrose agar were added to similar chambers; while to a third set distilled water was added. On the following day copper determinations were made on the

supernatant liquid after centrifuging. In the experiments with spores, from 2 to 4 p.p.m. of copper were found. The sterile agar gave from 4 to 7 p.p.m. copper, while the water checks gave 0.2 p.p.m.

From these experiments it is clear that the washings from sterile tubes of agar are capable of rendering the copper in Bordeaux soluble, and it is therefore necessary to adopt a method of obtaining the spores uncontaminated with material from the medium itself.

In subsequent experiments the fungi were cultured in petri dishes and the spores were obtained by suction applied from the laboratory vacuum line, through a pipette with an opening 1.5 to 2.0 mm. in diameter. In

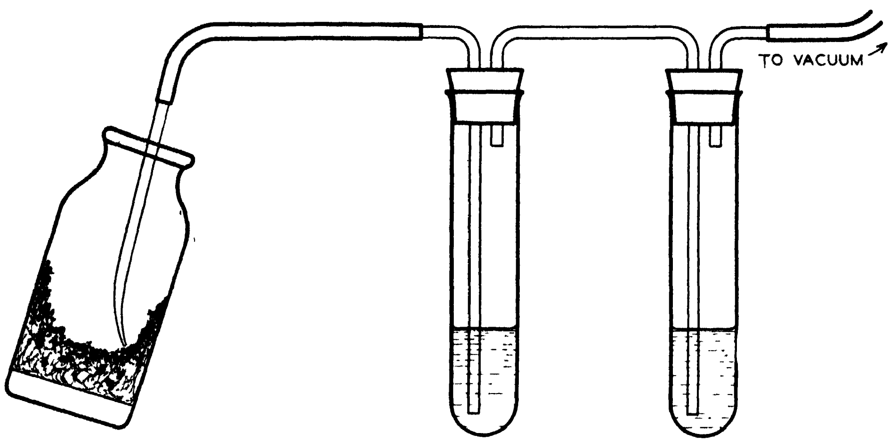


FIGURE 1. Vacuum apparatus for obtaining spores free from contamination by the medium.

this way masses of conidia were detached from the conidiophores and were caught in water traps inserted in the line as shown in Figure 1. Care was taken to avoid touching the surface of the agar. *Neurospora sitophila* will produce an abundance of conidia at an unusual height above the medium. Thus it was found desirable to culture this fungus in wide-mouth bottles of 200 cc. capacity (Fig. 1). Under these conditions the spores are produced several centimeters above the surface of the agar and danger of contamination from the medium becomes negligible. The spores of *Uromyces caryophyllinus* were of necessity obtained directly from the infected leaves. All of the water suspensions of spores obtained by the vacuum method were filtered through several layers of cheesecloth to remove aggregates of spores and, in the case of *N. sitophila*, *Sclerotinia fructicola*, and *Botrytis paeoniae*, occasional pieces of conidiophores. This method of obtaining the spores apparently caused no appreciable injury since the spores germinated as well as those obtained by the more customary method of washing.

Even when this vacuum technique was used, the spore filtrates were found to be capable of bringing into solution considerable amounts of copper. Accordingly a quantitative study was made of the relation between solubilizing action of spore filtrates, amounts of spore excretion, and the sensitivity to Bordeaux mixture of the seven fungous species mentioned above.

SOLUBILIZING ACTION OF DIFFERENT SPECIES

Spores from the different species of fungi were obtained by the method described above and suspended in 50 cc. of water for 5 hours. The concentration of spores was determined by microscopic counts on random samples from the spore suspension. The average standard deviation expressed as percentage of the mean was 8 per cent.

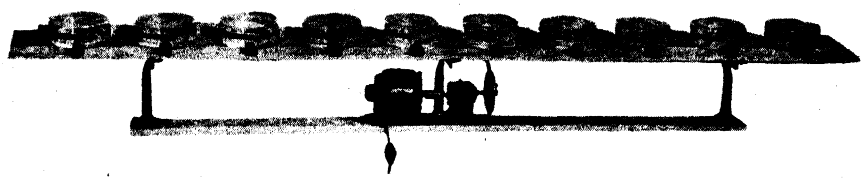


FIGURE 2. Agitator for solubility determinations. The rack is rocked on its long axis by the motor which by means of a speed reducer and crank arm produces about 45 oscillations per minute. The rubber bands around the inner moist chamber prevent excessive movement of the cover.

The spore suspension was filtered through Whatman No. 42 filter paper, made up to 50 cc. and placed in 20 cm. moist chambers. These chambers had been previously sprayed with 4–4–50 Bordeaux mixture and dried for at least 3 hours.

The chambers were placed on a specially constructed rack (illustrated in Fig. 2) which could be agitated mechanically in order to ensure equilibrium between the solution and the Bordeaux precipitate. The solutions were agitated for 16 hours at the temperature of the laboratory, between 21° to 25° C.

At the end of this period the suspensions were centrifuged at 2400 r.p.m. for 7 minutes. The dissolved copper was determined in 10 cc. samples pipetted from the supernatant liquid by the colorimetric method of Callan and Henderson (3) referred to above. Tests performed on the residual Bordeaux mixture adhering to the chambers indicated that there was an excess of undissolved copper.

The results of these experiments on the solubilizing action of spore filtrates are shown in Figures 3 and 4. The amount of copper brought into solution for a given species is directly proportional to the number of spores

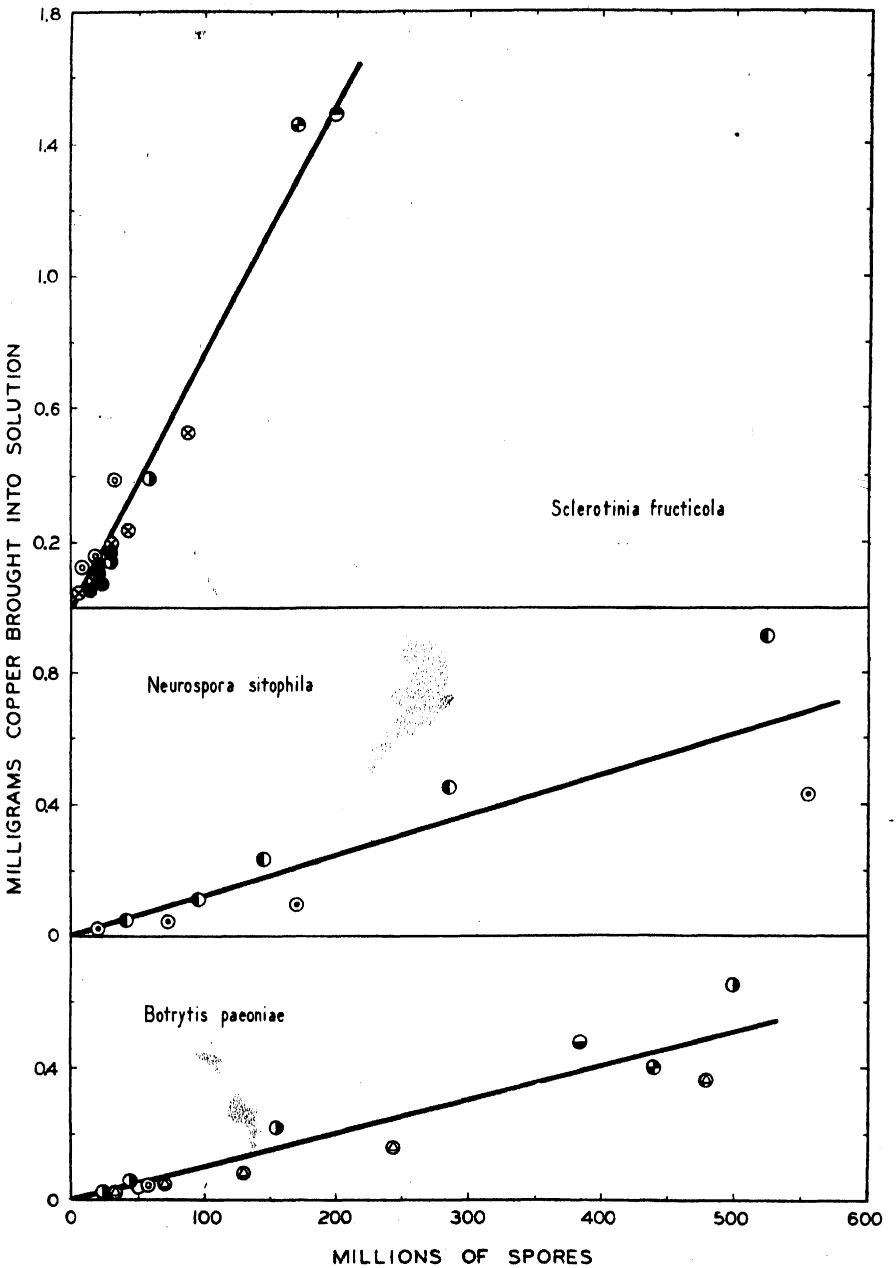


FIGURE 3. The solubilizing action of water extracts from the spores of *Sclerotinia fructicola*, *Neurospora sitophila*, and *Botrytis paeoniae* on dried Bordeaux mixture. Different kinds of dots indicate experiments performed at different times.

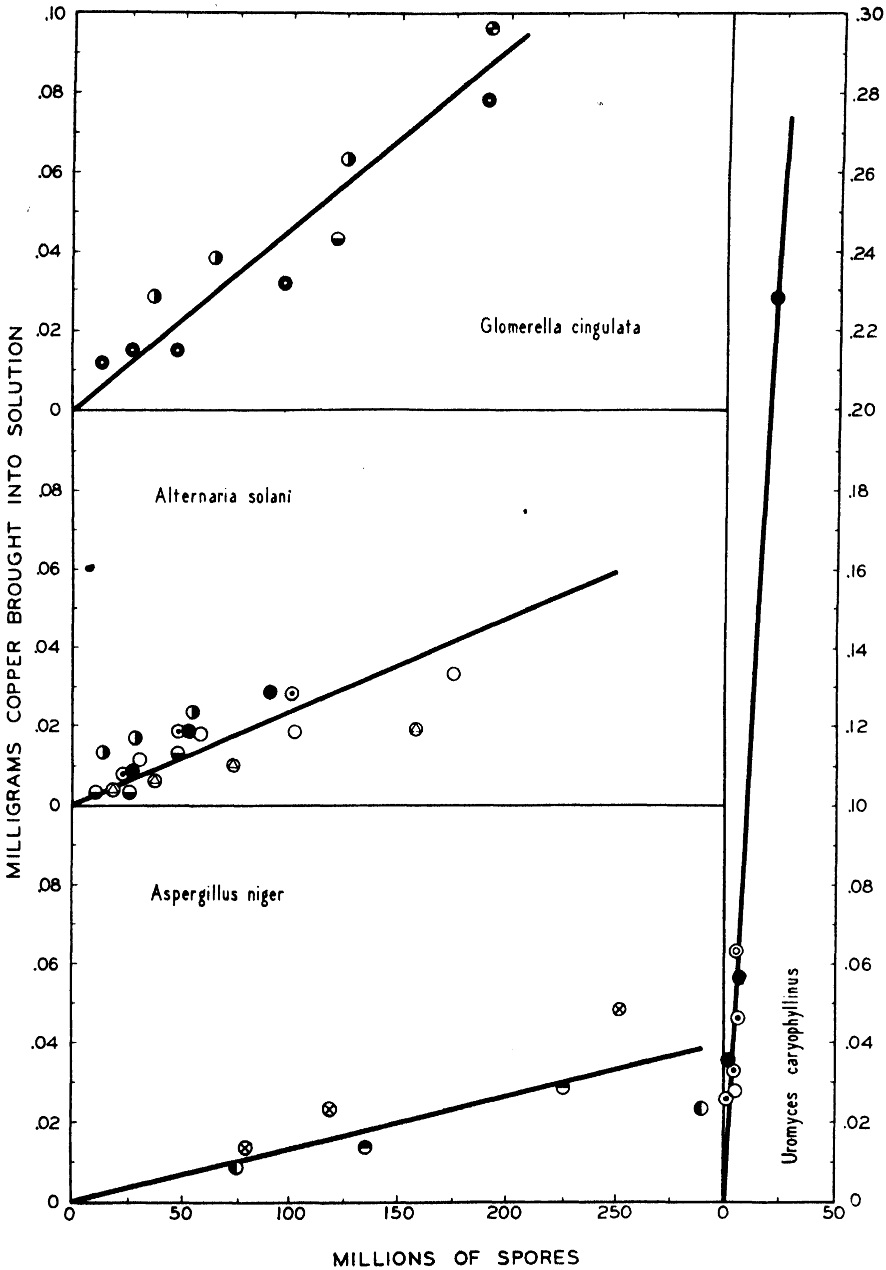


FIGURE 4. The solubilizing action of water extracts from the spores of *Glomerella cingulata*, *Alternaria solani*, *Aspergillus* sp., *niger* group, and *Uromyces caryophyllinus* on dried Bordeaux mixture. Different kinds of dots indicate experiments performed at different times.

used in preparing the spore filtrate. However, the ability of various species to bring copper into solution varies widely.

The age of the spores does not appear to influence the solubilizing action. Spores of *Sclerotinia fructicola* from five cultures varying from 6 to 60 days old did not differ significantly in their ability to bring copper into solution as is shown by the solid black dots in Figure 3. The kind of water also did not appear to be a factor since rain water produced the same result as distilled water.

Determinations were made of the total solids in the spore filtrates from the different species by evaporating to dryness measured volumes of spore filtrate derived from a known number of spores. Here also it was found that the amount of solids per given number of spores varied considerably for different species (Table I).

In general it was found that the spores of those species which excreted the largest amount of solids were also the ones which were most active in bringing copper into solution.

COMPARATIVE SENSITIVITY OF DIFFERENT SPECIES TO BORDEAUX MIXTURE AND TO COPPER SULPHATE

In order to determine whether the susceptibility of a given species to Bordeaux mixture is related to the solubilizing action of that species, comparative toxicity experiments were performed. Such comparative toxicity experiments present difficulties; for example the spores of certain species do not germinate well in the absence of some stimulant such as orange juice (15). However, orange juice itself will dissolve copper from Bordeaux and hence cannot be used in this case.

The question arises also whether Bordeaux mixture of varying concentrations should be prepared by diluting the original concentrated sample, or by keeping the lime constant and varying the concentration of copper. When an original concentrated Bordeaux suspension is highly diluted the particles of Bordeaux precipitate are no longer distributed uniformly through the solution. Also it is very probable that the composition of the original Bordeaux precipitate is changed by extreme dilutions, as it is known to be by prolonged washing. For these reasons the second method was adopted in the following experiments. The toxicity experiments were performed as previously described (6, 8, 9, 16). The sprayed slides were dried for at least six hours.

The results of these toxicity tests are shown in Table I, in which the figures in column 2 indicate the concentration of copper in the Bordeaux spray, which is required to prevent the germination of 90 per cent of the spores. Column 3 gives the same value for copper in the soluble form of copper sulphate.

In Table I is also shown the relative solubilizing action of spores of

the different species as well as the relative amount of material excreted by the spores. It may be seen that the species which have a high solubilizing action toward Bordeaux are those which are most susceptible to its toxic action. While there is wide difference in susceptibility to Bordeaux mixture among the different species, this is not true in the case of copper sulphate. This fact supports the hypothesis that it is not the total amount of copper which is important but the amount rendered soluble. In general also the species showing the highest solubilizing action are those which excrete the greatest amount of material.

TABLE I

COPPER SENSITIVITY, SOLID CONTENT, AND SOLUBILIZING ACTION OF WATER EXTRACTS ON BORDEAUX MIXTURE PER 100,000,000 SPORES

Fungous species	Mg. Cu per l. for LD 90		Mg. solids excreted	Mg. Cu brought into solution from Bordeaux by spore water extract
	Bordeaux mixture	Copper sulphate		
Uromyces carophyllinus	180	1.74	80.8	1.01
Sclerotinia fructicola	120	1.20	12.8	0.76
Botrytis paeoniae	390	2.23	2.78	0.10
Glomerella cingulata	500	1.40	2.14	0.046
Alternaria solani	2400	6.72	5.53	0.013
Neurospora sitophila	—	—	2.42	0.120
Aspergillus niger	—	—	0.44	0.023

WASHING EXPERIMENTS

The question arises as to whether the solubilizing material is continuously excreted by the spores, or whether it may be completely removed from them by washing without subsequent renewal.

In order to throw light on this question washing experiments were performed on the spores in the following manner. The spores were collected by the vacuum technique described above and were diluted to 50 cc. and allowed to stand for a definite time, usually one hour. The suspension was centrifuged, and the supernatant liquid decanted, water being added to the spores to make the volume 50 cc. This procedure was repeated, the spores being permitted to remain in the solution for varying periods. The washings were placed over dried Bordeaux mixture and after agitating overnight copper determinations were made.

Typical experiments are shown in Figure 5. The greater part of the solubilizing material is removed in the first washing and not readily renewed subsequently in most of the species studied. In the case of *Neurospora sitophila*, however, the fourth washing obtained after the spores had stood overnight gave about one-third the amount present in the water of the first washing.

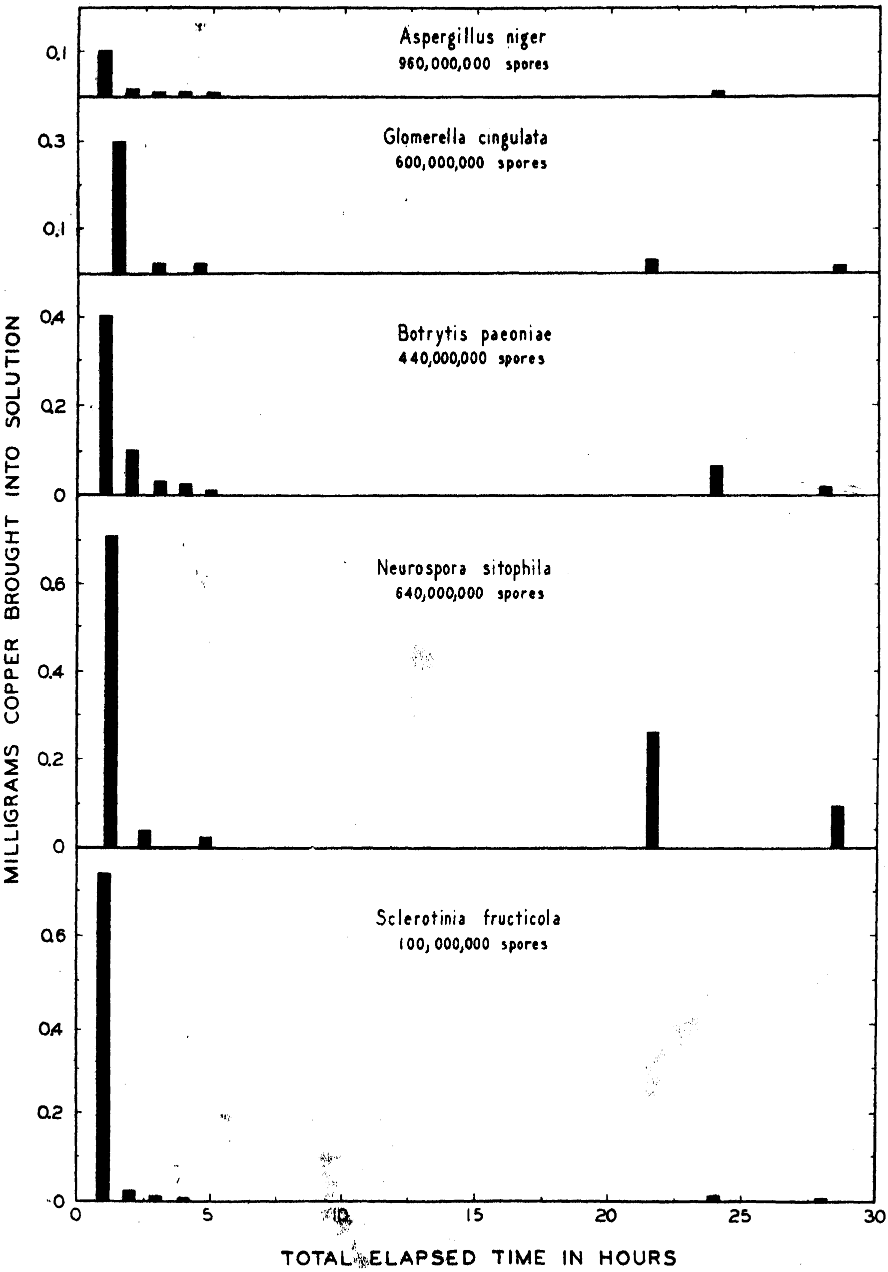


FIGURE 5. The effect of washing on the solubilizing action. The spores are suspended in water for definite periods of time, centrifuged, supernatant water extract decanted, tested for solubilizing action, spores resuspended in original volume let stand for a second period, etc.

NATURE OF THE ACTIVE SUBSTANCE

The material obtained by evaporation of the filtrate from spore suspensions was obviously a complex mixture. Qualitative tests showed the presence of sugars, proteins, and organic acids. The active substance or substances were shown to be in true solution by means of ultrafiltration experiments. Evaporation on the steam bath did not destroy the active principle. Substances which might be expected to bring copper into solution from alkaline mixtures such as Bordeaux include hydroxy acids such as citric, malic, tartaric, etc., as well as the amino acids. The ability of salts of hydroxy acids to form soluble complexes with copper oxide is exemplified by the case of Fehling's solution, used in determinations of reducing sugars. It is also well known that certain amino acids form soluble complexes with copper oxide and an analytical method for determining amino acids has been based on this fact (5).

The problem of identifying the substance or substances responsible for bringing the copper into solution is rendered difficult by the small amount of spore excretion available. Spores were collected daily from groups of 48 bottles containing two-weeks-old cultures of *Neurospora sitophila*. When about 360,000,000,000 spores had been obtained, the excretions were considered sufficient for a chemical examination. The concentrated solution of spore excretion was treated with neutral lead acetate giving a precipitate. This precipitate consisted partly of lead salts of organic acids and was decomposed with hydrogen sulphide and the liberated acids converted to ethyl esters as described by Birkinshaw and others (2) in their comprehensive investigation of the biochemistry of microorganisms. The small amount of esters obtained, about 0.75 cc., was submitted to fractional distillation in a miniature distilling flask in vacuo. The first fraction obtained gave with hydrazine hydrate a crystalline hydrazide which melted at a temperature of 177.5° C., indicating malic hydrazide. A mixed melting point with an authentic sample of malic hydrazide gave no depression. The amount of material available was insufficient for chemical analysis, but the presence of malic acid is indicated by the melting point and the appearance of the hydrazide obtained.

Hydrogen ion determinations of the spore excretions from *Sclerotinia fructicola*, *Botrytis paeoniae*, and *Neurospora sitophila* gave pH values, respectively, of 6.55, 6.40, and 6.40. Hence the malate excretion cannot act because of any acidic properties. Separate experiments have shown that neutral sodium malate will dissolve copper from dried Bordeaux mixture. When 500 p.p.m. of sodium malate were placed over dried Bordeaux as in the experiments with the spore excretions, 42.4 p.p.m. of copper were brought into solution. The compound formed by the action of malic acid salts on copper hydroxide has been shown by Wark and coworkers (10, 14) to be sodium cuprimalate. The amino acids, glycine and aspartic, were

also found to dissolve copper from Bordeaux mixture. At a concentration of 500 p.p.m., the two acids dissolved respectively 26.4 and 28.8 p.p.m. copper.

Recently a quantitative method for the determination of malic acid in plant material has been developed by Pucher, Vickery, and Wakeman (11). This method depends on the fact that malic acid may be oxidized by potassium permanganate in the presence of potassium bromide to give a bromine compound volatile with steam. This substance reacts with dinitrophenylhydrazine giving an insoluble product which is filtered off and dissolved in pyridine. The diluted pyridine solution on adding alkali develops a blue color proportional to the amount of malic acid. The method is applicable for small quantities (0.1 to 2.5 mg.) and hence appeared suitable for our use. When a weighed sample of the solid matter from spore filtrates of *Neurospora sitophila* was brought to pH 1 as described by Pucher, Vickery, and Wakeman (11), extracted with ether and the determination of malic acid performed as described by these authors, 3.1 per cent of malic acid was found.

Another sample of spore filtrate from the same fungus was evaporated on the steam bath until the content of solid matter was 7 per cent. Alcohol was added until the content of the solution was 80 per cent alcohol. The filtrate was evaporated to dryness in vacuo and determinations of the amino nitrogen were made by the Van Slyke method on the residue. The content of amino nitrogen found was 0.75 per cent of the solid matter in the original spore filtrate.

Thus it appears that malic acid (and perhaps other hydroxy acids) and in addition amino acids are found in the filtered solution in which spores of *Neurospora sitophila* have been suspended.

TOXICITY OF SODIUM CUPRIMALATE AND OF THE COPPER GLYCINE DERIVATIVE

Since it has been shown that these compounds can be formed by the action of spore filtrates on Bordeaux mixture the question arises as to their toxicity to fungous spores. Accordingly tests were performed in which sodium cuprimalate was compared with copper sulphate. A solution of the copper glycine derivative was obtained by agitating a solution of glycine (500 p.p.m.) with an excess of cupric oxide. This solution after filtering off the excess oxide was also compared with the copper sulphate solution both in distilled water and in 0.02 per cent filtered orange juice. The results of typical tests are illustrated in Figure 6 and show that copper in these forms exerts substantially the same toxic action as in the form of copper sulphate.

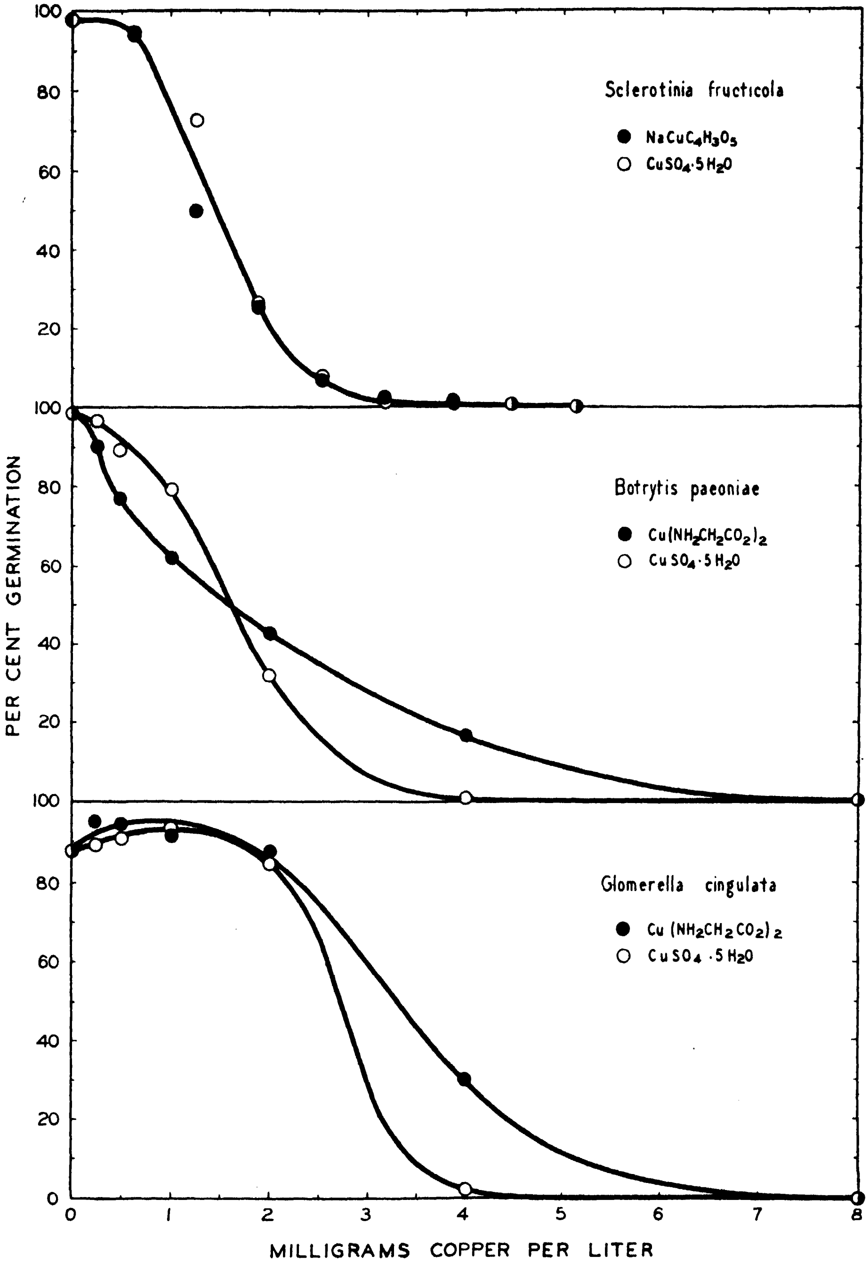


FIGURE 6. The comparative toxicity of sodium cuprimalate, of the copper glycine derivative, and of copper sulphate. All tests performed in 0.02 per cent filtered orange juice.

SUMMARY

1. By employing recent and extremely sensitive chemical tests for copper, a study has been made on the action of fungous spores in bringing copper into solution from Bordeaux mixture.

2. In the mother liquor above freshly prepared 4–4–50 Bordeaux mixture there was found about 1 p.p.m. of soluble copper. However, the copper that goes into solution in distilled water in contact with dried Bordeaux was found not to exceed 0.3 p.p.m., an amount insufficient to affect materially the germination of most fungous spores.

3. Fungous spores obtained by a vacuum technique which prevented their contamination by the nutrient medium, were suspended in water, allowed to stand for several hours and filtered. The filtrates were placed over dried Bordeaux mixture, and agitated overnight. The amount of copper rendered soluble varied with the species and was directly proportional to the number of spores. The water extracts from 100,000,000 spores brought into solution copper as follows: *Uromyces caryophyllinus* 1.01 mg., *Sclerotinia fructicola* 0.76, *Neurospora sitophila* 0.12, *Botrytis paeoniae* 0.10, *Glomerella cingulata* 0.046, *Aspergillus niger* 0.023, and *Alternaria solani* 0.013.

4. Determinations of total solids excreted by the spores showed that those species excreting the greatest amount of solids were also most active in bringing copper into solution from Bordeaux mixture.

5. Comparative toxicity tests of soluble copper (copper sulphate) and insoluble copper (Bordeaux mixture) showed, with the five fungi tested, little difference in sensitivity to copper sulphate and considerable difference to Bordeaux mixture. Furthermore those spores most sensitive to the toxic action of Bordeaux mixture were in general the ones most active in bringing copper into solution from Bordeaux.

6. The active material in the spore excretions is in true solution as shown by ultrafiltration tests. Practically all of the active material may be removed in the first washing of the spores and is not appreciably renewed except in the case of *Neurospora sitophila*.

7. By collecting 360,000,000,000 spores of *Neurospora sitophila* a quantity of spore excretion was obtained. From the lead precipitate of this, malic acid was identified by the melting point of its hydrazide. A mixed melting point with an authentic sample showed no depression. Quantitative determinations by the method of Pucher, Vickery, and Wakeman (11) indicated 3.1 per cent malic acid present in the solid matter excreted by the spores.

8. Amino nitrogen determinations of the solids excreted by spores of *Neurospora sitophila* showed 0.75 per cent amino nitrogen thus indicating the presence of amino acids.

9. Spore excretions are practically neutral, hence cannot act because of any acidic properties. It is well known that certain amino acids form

soluble complexes with copper oxide (5). Also it has been found that glycine and aspartic acid as well as neutral sodium malate will dissolve large amounts of copper from Bordeaux mixture.

10. Comparative toxicity tests of sodium cuprimalate and of a copper glycine derivative indicated that these forms of copper exert substantially the same action as copper sulphate.

11. It is believed that the salts of hydroxy acids such as malate and perhaps others, as well as of amino acids present in the spore excretions, act on Bordeaux mixture to form soluble toxic copper hydroxy and copper amino salts.

LITERATURE CITED

1. BARKER, B. T. P., and C. T. GIMINGHAM. The fungicidal action of Bordeaux mixtures. Jour. Agric. Sci. **4**: 76–94. 1911.
2. BIRKINSHAW, JOHN HOWARD, JOHN HENRY VICTOR CHARLES, ARTHUR CLEMENT HETHERINGTON, and HAROLD RAISTRICK. Studies in the biochemistry of micro-organisms. Part IX.—On the production of mannitol from glucose by species of *Aspergillus*. Phil. Trans. Roy. Soc. London B. **220**: 153–171. 1931.
3. CALLAN, THOMAS, and J. A. K. HENDERSON. A new reagent for the colorimetric determination of minute amounts of copper. Analyst **54**: 650–653. 1929.
4. HOCKENYOS, GEORGE L. Solubility of Bordeaux. Phytopath. **21**: 231–234. 1931.
5. KOBER, PHILIP ADOLPH, and KANEMATSU SUGIURA. A micro-chemical method for the determination of α- and β-amino acids and certain derivatives; in proteolysis, blood and urin. Jour. Amer. Chem. Soc. **35**: 1546–1584. 1913.
6. McCALLAN, S. E. A. Studies on fungicides. II. Testing protective fungicides in the laboratory. New York [Cornell] Agric. Exp. Sta. Mem. 128: 8–24. 1930.
7. ——— Studies on fungicides. III. The solvent action of spore excretions and other agencies on protective copper fungicides. New York [Cornell] Agric. Exp. Sta. Mem. 128: 25–79. 1930.
8. McCALLAN, S. E. A., and FRANK WILCOXON. The fungicidal action of sulphur. II. The production of hydrogen sulphide by sulphured leaves and spores and its toxicity to spores. Contrib. Boyce Thompson Inst. **3**: 13–38. 1931.
9. ——— Fungicidal action and the periodic system of the elements. Contrib. Boyce Thompson Inst. **6**: 479–500. 1934.
10. PACKER, JOHN, and IAN WILLIAM WARK. Cupritartrates. Jour. Chem. Soc. **119**: 1348–1355. 1921.
11. PUCHER, GEORGE W., HUBERT BRADFORD VICKERY, and ALFRED J. WAKEMAN. Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acids. Indus. & Eng. Chem. Anal. Ed. **6**: 288–291. 1934.
12. QUARTAROLI, A. Determination of manganese, copper and iron in minimum quantities and its application to biological research. Ann. Chim. Applic. **17**: 361–375. 1927. (*Abstr. in* Chem. Abstr. **22**: 927–928. 1928.)
13. THOM, CHARLES, and MARGARET B. CHURCH. The Aspergilli. 272 pp. Williams & Wilkins Co., Baltimore. 1926.
14. WARK, ELSIE EVELYN, and IAN WILLIAM WARK. Metallic hydroxy-acid complexes. Part V. Neutral a-cupritartrates. Jour. Chem. Soc. **1930**: 2474–2478. 1930.
15. WILCOXON, FRANK, and S. E. A. McCALLAN. The stimulation of fungus spore germination by aqueous plant extracts. Phytopath. **24**: 20. 1934.
16. ——— Fungicidal action of organic thiocyanates, resorcinol derivatives, and other organic compounds. Contrib. Boyce Thompson Inst. **7**: 333–339. 1935.
17. WILTSHIRE, S. P. The foundation species of *Alternaria* and *Macrosporium*. Trans. Brit. Mycol. Soc. **18**: 135–160. 1933.

EFFECT OF LOW TEMPERATURE IN SHORTENING THE HIBERNATION PERIOD OF INSECTS IN THE EGG STAGE

FLORENCE FLEMION AND ALBERT HARTZELL

Buds of woody plants of the temperate zone become dormant in early fall and do not resume growth until they have been subjected to a period of low temperature. The required time at low temperature varies greatly with different species. Certain seeds (4, 7), bulbs (5), tubers, and other plant parts are also dormant and some require low temperature preceding the resumption of growth. This suggested the possibility that insects of the colder temperate zone region which hibernate in the egg stage may also respond to a low temperature cycle. The results reported in this paper are believed to be of general biological interest in that eggs of certain species of insects respond similarly to low temperature as do certain plants and seeds.

It has been observed (6, p. 424) that severe and steady winters caused eggs of the Rocky Mountain locust to hatch more regularly in the spring than did a mild and changeable winter. Carothers (3) placed eggs of the grasshopper, *Circotettix*, in sand and kept them out-of-doors. These eggs when brought in late in January hatched within 2 to 4 weeks. Bodine (2) found that eggs of the grasshopper, *Melanoplus differentialis* (Thomas), after 39 days of winter would hatch at 23° C. in 40 days, while those which had not been exposed to low temperature hatched in 89 days.

MATERIAL AND METHODS

Egg-masses of the Eastern tent caterpillar, *Malacosoma americana* (Fabr.), fall cankerworm, *Alsophila pometaria* Harris, and the Chinese praying mantis, *Paratenodera sinensis* (Saussure), were collected at various intervals, from time of laying to spring in the vicinity of Yonkers, New York. The eggs of the Eastern tent caterpillar are laid in July in masses of about 300, principally on the twigs of wild cherry. The masses are about an inch in length. They encircle the twig and are covered with a gelatinous-like substance which gives a varnished appearance on hardening. The eggs of the fall cankerworm are laid in the fall by the wingless females in masses containing 50 to 100. The eggs are ash-gray in color and are deposited in straight rows mainly on the twigs of elm, maple, and other trees. The light brown egg-masses of the Chinese praying mantis are laid in October on twigs of low shrubs and contain from 200 to 300 eggs.

The eggs of the above species when collected were immediately placed in closed glass containers. They were subjected to various constant temperatures (for general description of temperature control see 9, p. 14) and

were transferred at definite intervals to room temperature (about 22° C.). With one exception no attempt was made to control the humidity in these experiments.

RESULTS

EASTERN TENT CATERPILLAR

On October 10, 1934, over 200 egg-masses of the Eastern tent caterpillar were collected and divided into lots of four, placed in closed one-quart mason jars and transferred to various constant temperatures as shown in Table I. After periods of 2, 4, 6, 8, 12, 16, 20, and 45 weeks they were transferred to room temperature. No larvae hatched at the higher temperatures (25°, 30°, and 35° C.) either before or after they were transferred to room temperature. It is clearly shown in Table I that there is a

TABLE I

EFFECT OF VARIOUS TEMPERATURES UPON SUBSEQUENT HATCHING OF EGGS OF EASTERN TENT CATERPILLAR*

Period at various temp., weeks	Av. No. larvae hatched from 4 egg-masses at room temp. after various periods at different temp.							
	1° C.	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
6	57	22	0	0	0	0	0	0
8	139	133	78	0	0	—	—	—
12	197	200	242	5	0	—	—	—
16	251	170	219	125	0	0	0	—
20	—	204	—	—	1	—	—	—
45	—	0	—	—	—	—	—	—

* Experiment started October 10, 1934.

marked increase, as the temperature is lowered, in the number of larvae hatched when subsequently transferred to room temperature. In the above experiments no attempt was made to control the humidity either before or after the transfer to room temperature. However, in another experiment no larvae hatched from egg-masses kept constantly at room temperature in atmospheres of various relative humidities ranging from no moisture to about 90 per cent humidity.

Twelve weeks at 1°, 5°, and 10° C. produced a high average of hatching when transferred to room temperature, while 15° C. only produced an average of 5, and none were hatched at 20° C. The figures in Table I represent averages of four egg-masses. For periods shorter than 12 weeks, for example 8 weeks, higher averages were obtained at 1° and 5° C. than at 10° C., while at 15° C. none hatched. After six weeks no larvae hatched from 10° or 15° C., while a few were obtained from 1° and 5° C. Periods shorter than six weeks produced no hatching at any temperature used in

this experiment, while a long period such as 45 weeks at 5° C. was deleterious. It is evident that the low temperatures shown in Table I are efficacious in producing a large number of hatches after the eggs are transferred to room temperature. The out-of-doors conditions are also favorable in bringing about the hatching of larvae of the eggs when brought into room temperature, after the winter has advanced sufficiently to correspond to our experimental low temperature. Egg-masses collected in October, 1934, and kept continuously at room temperature did not hatch, while egg-masses collected late in January, 1935, hatched an average of 207 larvae per egg-mass.

Preliminary tests made from eggs collected in July, 1935, immediately after they were laid and subjected to various temperatures, gave a very low number of hatches. While a few hatched after two weeks at 1° and 5°

TABLE II

RATE OF HATCHING OF EASTERN TENT CATERPILLARS FROM EGGS WHICH WERE SUBJECTED TO DIFFERENT PERIODS OUT-OF-DOORS

Date of transfer, out-of-doors to room temp.	Av. No. of larvae per egg-mass hatched at room temp.*	Days required to reach 50% total hatch at room temp.
Nov. 15, 1935	0	—
Dec. 11, 1935	86	37
Dec. 26, 1935	122	24
Jan. 14, 1936	256	16
Jan. 30, 1936	232	16
Feb. 15, 1936	191	10
Feb. 26, 1936	241	9
Mar. 17, 1936	270	3.5
Apr. 1, 1936	Hatching out-of-doors	

* These figures represent averages of 8 egg-masses.

C., no increase in the number of hatches was obtained with longer periods at either the low or high temperatures. However, eggs collected in the fall of the same year responded similarly to the results of 1934 as shown in Table I.

The low temperature treatment not only affects the number of hatches obtained but also affects the rate of emergence when transferred to room temperature. The time required for these hatchings decreased as the period of low temperature increased. Thus in the 5° C. series shown in Table I (in which only total hatches are given) it required two months for a total of 22 to hatch from the six-week lot, while a total of 200 from the 12-week lot was obtained in six weeks, and only four days were required for a total of 204 from the 20-week lot.

The rate of emergence is clearly shown in the experiment presented in Table II. Collection made at intervals throughout the winter of 1935 were placed at room temperature. Eggs collected on November 15 failed to

hatch, while those collected on December 11 produced an average of 86 larvae per egg-mass hatching over a period of 23 to 48 days. Material brought in on March 17, 1936, gave an average of 270 larvae per egg-mass in three to eight days. It can be seen in Table II that as the winter advances the time required to reach 50 per cent of the total number of hatches progressively decreases. December 1935 was unusually cold with a mean temperature approximately 4.5° F. below the 67-year average for the nearest weather station. The minimum temperature of this winter was −3° F. on January 22, 1936 (8, 10).

Application to ovicidal tests. The above experiments show that eggs of Eastern tent caterpillar do not hatch unless given a pre-treatment at low

TABLE III

EFFECT OF VARIOUS TEMPERATURES UPON SUBSEQUENT HATCHING OF EGGS OF FALL CANKERWORM*

Temperature, °C.	Percentage of larvae hatched when transferred to room temperature after exposure to different temp. for following weeks					
	3	6	9	12	15	18
−16	1	—	0	—	0	—
1	0	27	14	32	15	35
5	5	22	36	18	47	12
10	29	21	45	57	40	13
15	12	11	5	15	25	47
20	2	11	4	9	6	0
Room temp. (about 22)	0	0	3	9	1	2
Out-of-doors (winter)	—	—	33	44	56	28

* Experiment started December 13, 1935.

temperature. In order to adequately test the ovicidal action on these eggs, they must be exposed to low temperature either before or after the applications are made. If the eggs are not subjected to low temperature, the failure to hatch may not be due to the toxicity of the ovicide but due to the physiological condition of the eggs.

Four lots of egg-masses collected in the fall of 1935 were placed at 5° C. for 12 weeks and then transferred to room temperature. One lot had been dipped in an ovicidal solution prior to being subjected to low temperature to represent a fall spraying. Another lot was dipped after a period of 12 weeks at 5° C. to represent a spring spraying. The remaining two lots were used as controls. The number of hatches obtained from the eggs treated by the ovicide as compared with the hatches from the controls showed whether the solution tested was an effective ovicide. Eggs collected in the spring of 1936 and dipped as above were not given a period at 5° C. because the pre-treatment had occurred under natural conditions.

FALL CANKERWORM

Egg-masses of the fall cankerworm were collected December 13, 1935, immediately after being laid, and lots of 150 eggs were placed in corked test tubes of 50 cc. capacity. These were placed at the various temperatures shown in Table III. At three-week intervals, they were transferred to room temperature. On the same dates that these transfers were made eggs collected out-of-doors were also brought to room temperature. While a low percentage hatched from the 20° C. and 15° C. pre-treatments, a much higher percentage was obtained from 10°, 5°, and 1° C. The eggs from out-of-doors gave similar results to those from 1°, 5°, and 10° C. It is interesting to note that −16° C. was ineffective.

CHINESE PRAYING MANTIS

Egg-masses of the Chinese praying mantis collected in early December, 1935, were placed at 5° C. for various intervals followed by transfer to room temperature. After 3, 6, 9, and 12 weeks at 5° C. the hatching of the nymphs occurred within 27 to 36 days after the transfer to room temperature. The controls at room temperature also hatched within this period. From these preliminary results, there is no indication that the eggs of this species require a pre-treatment at low temperature.

DISCUSSION

The Eastern tent caterpillar is indigenous to North America and ranges east of the Rocky Mountains, north to the deciduous area of Canada, and south to the Gulf States. The active feeding stage of the larvae is timed with the development during spring and early summer of the leaves of the wild cherry, its principal food plant. Since the larvae hatch in spring from eggs laid the previous summer, there is either a mere delay in hatching or a required treatment of low temperature necessary for hatching. The above experiments show that they not only require a low temperature but the period at low temperature determines the rate and number of hatches when transferred to a higher temperature.

Eggs from out-of-doors brought to room temperature in late March of this year, hatched within 3 to 8 days and gave a very high average per egg-mass. This indicates that the conditions during the winter were very conducive to maximum hatching within a minimum time. This sudden appearance of great numbers makes proper control difficult. During some seasons the hatching is spread over a longer period of time thereby making control less difficult. This delay in hatching may be the result of a mild winter failing to give a maximum pre-treatment of low temperature.

This phenomenon of low temperature cycle is of importance in timing application of ovicides under laboratory conditions. Experiments conducted on tent caterpillar egg-masses collected in the spring have adequate

controls because the eggs are in a condition for hatching, while those collected in the fall are without adequate controls unless both the treated and the controls are given a period at low temperature.

Eggs of the fall cankerworm, another representative of the order *Lepidoptera,* also respond to the low temperatures. While the results in this case are not so specific as to low temperature requirement as the tent caterpillar, they show a response to a greater range of low temperature. Since the eggs of this species are laid very late in the fall (early December) and hatch in May, they do not receive under natural conditions as much accumulated low temperature as the eggs of the tent caterpillar which are laid much earlier (summer) and hatch in April. Very low temperature ($-16°$ C.) for fall cankerworm was ineffective while a long period (45 weeks) at 5° C. for the tent caterpillar was deleterious. This suggests that severe and prolonged winters may result in a high natural mortality.

The Chinese praying mantis, a representative of the order *Orthoptera,* is not indigenous but was accidentally introduced near Philadelphia from the Orient in about 1896 (1, p. 122). It ranges from subtropical regions northward and hence the eggs would not be likely to require a pre-treatment of low temperature which is consistent with our experimental results.

Some of the more temperate plants which survive in the north temperate region do so because the seeds which are shed in the fall do not then receive sufficient warm temperature to germinate. They are not injured in overwintering in the soil and merely remain there until favorable weather in the spring. The behavior of the eggs of the Chinese praying mantis may be a similar illustration among the insects since the eggs are laid in October and do not receive the minimum hatching temperature until in the spring.

The manifestation of a low temperature cycle in the eggs of the tent caterpillar may be very similar to the behavior found in certain seeds. Some seeds will never germinate unless given a period in a moist medium at low temperature during which chemical and physical changes occur in the imbibed seeds. The breakdown of fats and oils during this process spoken of as after-ripening in seeds is associated with a building up of sugars and amino acids and an increase in catalase and peroxidase activity. Our present plan is to determine whether similar changes occur in the eggs of the tent caterpillar which require a low temperature prior to hatching. Some seeds can be killed if kept too long at the favorable after-ripening temperature just as 45 weeks at 5° C. was too long for eggs of the tent caterpillar. Temperatures lower than 0° C. do not kill certain seeds but are merely unfavorable for the occurrence of after-ripening processes. This may explain why $-16°$ C. was ineffective for the eggs of the fall cankerworm.

SUMMARY

1. Egg-masses of the Eastern tent caterpillar, *Malacosoma americana*, collected in the fall, hatched at room temperature when given a pretreatment of eight to twelve weeks at 1°, 5°, and 10° C. The time required for hatching decreased as the period at low temperature increased. No hatching of larvae was obtained from those kept constantly at room temperature.

2. Eggs of the fall cankerworm, *Alsophila pometaria*, also respond to low temperature exposures; while a few hatch when kept constantly at room temperature (about 22° C.), a much higher percentage is obtained at room temperature when preceded by periods of three to eighteen weeks at 1°, 5°, 10°, and 15° C.

3. No low temperature treatment is required for the hatching of nymphs of the Chinese praying mantis, *Paratenodera sinensis*. However, egg-masses subjected for three to twelve weeks at 5° C. were not injured.

LITERATURE CITED

1. BLATCHLEY, W. S. Orthoptera of northeastern America with especial reference to the faunas of Indiana and Florida. 784 pp. Nature Publishing Company, Indianapolis. 1920.
2. BODINE, JOSEPH HALL. I. Effect of temperature on rate of embryonic development of certain *Orthoptera*. Jour. Exp. Zool. **42**: 91–109. 1925.
3. CAROTHERS, E. ELEANOR. Notes on the taxonomy, development and life history of certain Acrididae (*Orthoptera*). Trans. Amer. Ent. Soc. **49**: 7–24. 1924.
4. CROCKER, WM. Mechanics of dormancy in seeds. Amer. Jour. Bot. **3**: 99–120. 1916.
5. DENNY, F. E., and LAWRENCE P. MILLER. Storage temperatures and chemical treatments for shortening the rest period of small corms and cormels of gladiolus. Contrib. Boyce Thompson Inst. **7**: 257–265. 1935.
6. Effect of the weather on the eggs. *In* First annual report of the United States Entomological Commission for the year 1877 relating to the Rocky Mountain locust. 477 [295] pp. Government Printing Office, Washington. 1878.
7. FLEMION, FLORENCE. Physiological and chemical changes preceding and during the after-ripening of *Symphoricarpos racemosus* seeds. Contrib. Boyce Thompson Inst. **6**: 91–102. 1934.
8. NEW YORK METEOROLOGICAL OBSERVATORY OF THE DEPARTMENT OF PARKS. Report. **1935**: 83–192. 1936.
9. Temperature controls. *In* Contrib. Boyce Thompson Inst. **1**: 14–16. 1925.
10. UNITED STATES DEPARTMENT OF AGRICULTURE WEATHER BUREAU. Daily weather map. January 2–April 1. 1936.

THE DETERMINATION OF PYRETHRIN I[1,2]

FRANK WILCOXON

INTRODUCTION

Since the identification of the active principles of pyrethrum flowers by Staudinger and Ruzicka (11) several chemical methods have been devised for their determination in flowers and extracts. The earlier methods are discussed in the monograph by Gnadinger on pyrethrum flowers (2). They may be grouped under three headings as follows:

(1) Reduction methods
(2) Acid methods
(3) Condensation methods

Methods of the first group depend on the reducing power of the pyrethrins toward alkaline copper solutions as in the method of Gnadinger and Corl (3), or toward alkaline ferricyanide solution as in the method of Martin and Tattersfield (7). Methods of the second group depend on the fact that the pyrethrins are esters which may be saponified by alkali yielding the two acids, chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid. If these acids can be quantitatively separated from the reaction mixture and from each other they may be titrated. Recently a special method for pyrethrin II has been proposed by Haller and Acree (4) based on a determination of its methoxyl group. Methods of the third group include the formation of semicarbazones and the determination of the amount of the latter formed. Probably the methods most widely used, at least in this country, are the copper reduction method of Gnadinger, and the modification of the acid method introduced by Seil (10). Extensive investigations have made it clear that these methods give at least an approximate idea of the toxicity to be expected, but their application as methods of precision leaves much to be desired. Analysts in different laboratories often fail to check closely on a given sample, and in addition the methods lack specificity. Ripert (9) has noted objections to these methods and has suggested a new method which does not, however, overcome the sources of error mentioned in this paper.

The present paper contains a study of the Seil method for pyrethrin I as well as a new proposed method for pyrethrin I which appears to merit serious consideration on account of its specificity and ease of application.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 121.
[2] This article was preprinted July 28, 1936.

TEST OF SEIL METHOD FOR PYRETHRIN I

The various modifications of the acid method for pyrethrin I depend on the assumption that chrysanthemum monocarboxylic acid may be quantitatively removed by steam distillation from the acidified mixture of acids obtained by saponification of the pyrethrins, and that it may be extracted from the distillate with petroleum ether, uncontaminated by other acid substances. Staudinger and Harder tested the purity of the volatile petroleum ether soluble acid by conversion to the anilide. Harder points out, however, that the anilide crystallized badly (5). Tattersfield, Hobson, and Gimingham (13) studied the steam distillation of the pyrethrin acids and reported that the distillate was acid even after prolonged distillation, although when a sample of the pure acid was used, 95 per cent came over in the first 20 cc. of distillate, and practically all was recovered in 40 cc. of distillate. Hence it was concluded that acids other than the monocarboxylic acid came over during the steam distillation, and these authors relied on a petroleum ether extraction of the distillate to separate the chrysanthemum monocarboxylic acid from these other acids.

In order to study the behavior of the monocarboxylic acid on steam distillation, a sample of this acid was prepared in the following manner: 188 g. of a commercial petroleum ether extract of pyrethrin flowers containing 10.38 per cent pyrethrin I (Seil method) were saponified with alcoholic NaOH in four portions.[3] The saponified mixtures were each treated with 100 cc. of 10 per cent $BaCl_2$ after dilution with water and boiling off the alcohol as in the Seil analytical method. The filtrates from the barium chloride treatment were acidified with H_2SO_4 and extracted twice with low boiling petroleum ether. The petroleum ether extracts were re-extracted with aqueous sodium hydroxide to remove neutral substances. The solution of sodium salts was then carefully acidified with H_2SO_4 and again extracted with petroleum ether. The final petroleum ether extracts were combined, washed free from sulphuric acid, dried with Na_2SO_4, and evaporated first on the steam bath and finally in vacuo. In this way 11.1 g. of crude acids were obtained as a yellow oil.

This material was separated into three portions by vacuum distillation at a pressure of 2.5 mm. The first fraction and the residue in the distillation flask were discarded. Fraction 2 melted at 15° to 16° C. and fraction 3 at 16° to 17° C. Fraction 2 was redistilled and the high boiling portion united with fraction 3.

This combined sample was refractionated yielding three fractions with the following analytical figures:

[3] The extract was furnished by S. B. Penick and Co., to whom the author wishes to make grateful acknowledgement for their cooperation.

Fraction	Weight	Boiling range at 2.5 mm.	M. Pt.	Monocarboxylic acid by alkali titration, per cent
1a	0.75 g.	111°–113° C.	17.2° C.	98.9
2a	3.2 g.	113°–115° C.	17.9° C.	99.3
3a	2.0 g.	115°–117° C.	18.1° C.	99.8

Fraction 3a was recrystallized in a refrigerated room from 80 per cent CH_3OH. The needle-like crystals melted at 18.2° C. Microchemical combustion yielded the following results: Calc. for $C_{10}H_{16}O_2$: C, 71.37; H, 9.58. Found: C, 71.26; H, 9.51. The silver salt was prepared by treating a neutralized solution of the acid with a slight excess of silver nitrate. The needle-like crystals were filtered, washed, and dried in a vacuum desiccator protected from the light. *Anal.* Subs., 0.1645: g. Ag, 0.0646. Calc. for $AgC_{10}H_{15}O_2$: Ag, 39.23. Found: Ag, 39.25.

A useful physical constant for deciding questions of identity and structure is the parachor of Sugden (12). This constant is a function of the density and surface tension of a liquid. The calculated value of the parachor may be obtained by summing up the atomic parachors given by Sugden with the necessary structural contributions in case the compound is unsaturated or contains ring structures. The value for chrysanthemum monocarboxylic acid calculated in this way is 396.2. The parachor as determined from measurements of density and surface tension on fraction 3a was 391. The difference, 1.3 per cent, is sufficiently small to indicate that this fraction consists of chrysanthemum monocarboxylic acid.

In order to determine the percentage recovery of the monocarboxylic acid, weighed portions were submitted to steam distillation in the presence of H_2SO_4 as described by Seil. The distillate was collected in two portions of 250 cc., each of which was separately extracted with two 50 cc. portions of low boiling petroleum ether. The washed petroleum ether extracts were titrated with N/50 barium hydroxide solution which had been standardized on Bureau of Standards benzoic acid, and on constant boiling HCl. In one experiment in which 26.3 mg. of the acid were submitted to steam distillation, the total recovery was 71.2 per cent. The titrated solution was returned to the still and again steam distilled as before, giving a recovery of 56.8 per cent of the amount taken initially. In another experiment in which 38.4 mg. of the acid were subjected to steam distillation, the total recovery on the first distillation was 68.8 per cent. On redistillation of the titrated sample 53.8 per cent recovery was obtained. A third distillation gave 41.9 per cent recovery. These results indicate that the Seil method must inevitably give low results for pyrethrin I, since about 25 per cent of

the acid appears to be lost in the distillation. Furthermore, the acidity of the distillate after the petroleum ether extraction is insufficient to account for the missing acid. The monocarboxylic acid is either not entirely volatile with steam, or it is partly destroyed by heating in the presence of the sulphuric acid in the still.

A useful reaction for investigations on the chrysanthemum monocarboxylic acid is the reaction with Denigés' acid mercuric sulphate reagent, discovered by Seil (10). When the acid is treated with an excess of the reagent at temperatures in the neighborhood of 20° C. a pink color develops resembling that of phenolphthalein. This color gradually changes to violet, and if the amount of the monocarboxylic acid used be sufficient, a crystalline precipitate of mercurous sulphate is deposited, usually somewhat contaminated with colored material. The color reaction appears to be highly specific for the monocarboxylic acid since it is not given by the dicarboxylic acid, nor by any other substance tested by the present author, even though there exist substances capable of reducing mercury under these conditions. The intensity of color developed is roughly proportional to the amount of mono-acid taken, but since the color is fugitive it does not lend itself to quantitative colorimetric measurement. The reduction of the mercury may, however, be followed quantitatively, and this was accomplished in the following manner. A neutralized solution of the monocarboxylic acid was brought to a volume of about 10 cc. and placed in a 100 cc. beaker. Ten cc. of Denigés' reagent were added and the solution allowed to stand for one hour. At the end of this period 3 cc. of saturated NaCl solution were added with stirring and after a few moments the solution was filtered and the precipitate washed several times with distilled water. The funnel with the filter paper was placed in the neck of a 150 cc. titrating bottle. With a glass stirring rod a hole was punched in the filter and the major portion of the precipitate was washed into the bottle, using not more than 20 cc. of water. The filter paper was then folded and added to the bottle. Thirty cc. of concentrated HCl were then added and 6 cc. of chloroform. The reduced mercury was titrated with M/100 KIO_3 as described by Jamieson (6) until the iodine color had disappeared from the chloroform. Under these conditions 3 atoms of mercury were reduced per mol. of chrysanthemum monocarboxylic acid used. Fraction 1a gave 2.83 atoms of Hg reduced per mol. of acid calculated as the monocarboxylic acid, fraction 2a, 3.00 atoms of Hg, and fraction 3a, 2.99 atoms of Hg reduced. There is usually a very slight correction for mercurous mercury in the Denigés reagent, but it does not amount to more than 1 or 2 drops of M/100 potassium iodate. Pure samples of chrysanthemum dicarboxylic acid do not reduce mercury under these conditions. Furthermore, samples of pyrethrin resins which had been submitted to saponification and steam distillation were tested for the mono-acid in the residue remaining in the

still. This residue was extracted with two 50 cc. portions of petroleum ether and the ether extracts were extracted with aqueous NaOH. The NaOH solution was acidified with a slight excess of H_2SO_4 and re-extracted with petroleum ether. The petroleum ether solutions were washed free of H_2SO_4, united, and neutralized with alkali. The final solution gave no color reaction with Denigés' reagent and only traces of calomel on standing one hour, with subsequent addition of NaCl solution. Apparently the color reaction of Seil and the reduction of mercuric sulphate are brought about by chrysanthemum monocarboxylic acid. Balbiano and Paolini (1) have studied the reducing action of substances containing the propenyl and allyl groups toward mercuric acetate, and find that propenyl compounds readily reduce mercuric acetate while the corresponding allyl compounds do not. According to Staudinger and Ruzicka (11) chrysanthemum monocarboxylic acid contains the homologous isobutenyl group, and it is probably to this fact that its reducing action may be ascribed.

In order to obtain additional evidence regarding the nature of the acid which is titrated in the Seil method for pyrethrin I, the following experiment was performed. A sample of pyrethrin resins was saponified and steam distilled, the distillate being extracted with low boiling petroleum ether. The petroleum ether solution was dried and the solvent removed in vacuo. A portion of the material isolated in this way was weighed out and titrated with N/50 barium hydroxide, and the result calculated to the monocarboxylic acid, giving 84.8 per cent. The titrated solution was then treated with 10 cc. of Denigés' reagent as previously described and the reduced mercury determined by iodate titration. The result indicated 57.6 per cent of the monocarboxylic acid, if we assume, as found previously, that 1 mol. of the pure acid reduces 3 atoms of mercury. A similar experiment performed on material from another pyrethrin sample gave 91.3 per cent acid by alkali titration and 63.1 per cent by the mercury reduction method. Apparently, then, the acid obtained by steam distillation is contaminated with acid substances other than the desired monocarboxylic acid.

We thus have two sources of error which have opposing effects on the analytical result by the Seil method. In the first place it has been shown by steam distillation of purified isolated monocarboxylic acid that only about three-quarters of the amount taken is recovered. In the second place indications have been obtained that the steam distillate is contaminated with acid material soluble in petroleum ether, which is not the monocarboxylic acid.[4] The net effect of these two sources of error appears to give rise to results for pyrethrin I which are somewhat too low. For example, a

[4] The fact that there is a loss of the monocarboxylic acid in the Seil method was confirmed by Mr. David Hoyer of the John Powell Co., who obtained a recovery of only 89.3 and 89.5 per cent on redistillation of two titrated samples by the Seil method.

sample of pyrethrin resins giving 10.38 per cent pyrethrin I by the usual Seil method, gave 13.16 per cent by the mercury method. Another sample of partly purified resins high in pyrethrin I gave 35.7 per cent by the Seil method and 39.9 per cent by the mercury method.

REMARKS ON THE MERCURY REDUCTION METHOD

The sample should be of such a size that it will contain between 50 to 70 mg. of pyrethrin I. The preliminary treatment is the same as that described by Seil. The 200 cc. aliquot obtained after the barium chloride treatment is treated in a separatory funnel with 1 cc. conc. H_2SO_4, and extracted with two 50 cc. portions of low boiling petroleum ether. The barium sulphate precipitate does not seem to cause any difficulty. The petroleum ether extracts are washed successively with several small portions of water, and filtered through a small plug of cotton into a fresh separatory funnel. The combined petroleum ether extract is shaken vigorously with a slight excess of N/10 NaOH and the aqueous layer is run off into a small 100 cc. beaker. The ether layer is washed once with a few cc. of water and the washings added to the beaker. The beaker containing about 10 to 12 cc. is treated with 10 cc. of Denigés' reagent (8, p. 490) and allowed to stand one hour. Three cc. of saturated NaCl solution are added after which the precipitated calomel (and possibly other substances) are filtered and washed several times. The precipitate and filter paper are introduced into a titrating bottle containing 20 cc. H_2O, 30 cc. conc. HCl, cooled, and 6 cc. chloroform added. The titration with M/100 KIO_3 is performed as described by Jamieson until the iodine color disappears from the chloroform. In the case of a very small sample a little ICl solution may be added as suggested by Jamieson. The end point is not entirely permanent, so the titration should be carried out promptly with vigorous shaking.

According to Jamieson 1 mol. of iodate is equivalent to 4 atoms of mercurous mercury, while we have found 3 atoms of mercurous mercury equivalent to 1 mol. of pyrethrin I. Hence the factor for converting cc. M/100 KIO_3 to pyrethrin I is:

$$1\text{ cc.}\frac{M}{100}\,KIO_3 = \frac{0.04}{3} \times 330 = 4.4\text{ mg. pyrethrin I}$$

Since a 200 cc. aliquot was taken out of 250 cc., this factor (4.4) must be multiplied by 1.25 to give mg. pyrethrin I in the sample.

SUMMARY

Chrysanthemum monocarboxylic acid has been isolated from a pyrethrin concentrate and its properties described.

Samples of the purified acid submitted to steam distillation as in the Seil method for pyrethrin I failed to give quantitative recovery of the acid.

When the same sample was repeatedly steam distilled a loss of the acid was demonstrated at each distillation.

The reaction of the monocarboxylic acid with Denigés' reagent was studied quantitatively by applying the iodate titration method of Jamieson to the reduced mercury. It was found that three atoms of mercury are reduced per molecule of mono-acid.

Examination of the steam distilled acid from a pyrethrin concentrate showed that it is contaminated by other acids which are included in the titration by the Seil method.

The net effect of these two sources of error is to cause the Seil method for pyrethrin I to give lower results than the true value.

The reaction of the monocarboxylic acid with Denigés' reagent has been adapted to the quantitative determination of pyrethrin I in flowers and extracts, and examples of the application of this method are given.

LITERATURE CITED

1. BALBIANO, L., und V. PAOLINI. Reactionen von Mercuriacetat gegenüber Terpenen und Verbindungen, die die Gruppe C_3H_5 enthalten. Ber. Deutsch. Chem. Ges. **36:** 3575–3584. 1903.
2. GNADINGER, C. B. Pyrethrum flowers. 269 pp. McLaughlin Gormley King Co., Minneapolis, Minn. 1933.
3. GNADINGER, C. B., and C. S. CORL. Studies on *Pyrethrum* flowers. I. The quantitative determination of the active principles. Jour. Amer. Chem. Soc. **51:** 3054–3064. 1929.
4. HALLER, H. L., and FRED ACREE, JR. Constituents of pyrethrum flowers. Determination of pyrethrin II. Indus. & Eng. Chem. Anal. Ed. **7:** 343–344. 1935.
5. HARDER, HUGO. Über die Bestimmung des wirksamen Bestandteiles des Insektenpulvers und über die Herstellung technisch haltbarer Pyrethrinlösungen. p. 46–79. Part 2 *of* Diss. Zurich. 1927.
6. JAMIESON, GEORGE S. Volumetric iodate methods. 96 pp. Chemical Catalog Co., New York. 1926.
7. MARTIN, J. T., and F. TATTERSFIELD. The evaluation of pyrethrum flowers (*Chrysanthemum cinerariaefolium*). Jour. Agric. Sci. **21:** 115–135. 1931.
8. Pharmacopoeia of the United States of America. 10th dec. rev. 626 pp. J. B. Lippincott Co., Philadelphia. 1926.
9. RIPERT, J. Sur un nouveau procédé d'analyse des produits contenant des extraits de pyrèthre. Ann. Fals. et Fraudes **28:** 27–38. 1935.
10. SEIL, HARVEY A. Estimation of pyrethrins. Soap **10** (5): 89, 91, 111. 1934.
11. STAUDINGER, H., und L. RUZICKA. Insektentötende Stoffe. I. Über Isolierung und Konstitution des wirksamen Teiles des dalmatinischen Insektenpulvers. Helvetica Chim. Acta **7:** 177–201. 1924.
12. SUGDEN, SAMUEL. The parachor and valency. 224 pp. George Routledge & Sons, Ltd., London. 1930.
13. TATTERSFIELD, F., R. P. HOBSON, and C. T. GIMINGHAM. Pyrethrin I and II. Their insecticidal value and estimation in pyrethrum (*Chrysanthemum cinerariaefolium*). I. Jour. Agric. Sci. **19:** 266–296. 1929.

RELATIVE TOXICITY OF PYRETHRINS I AND II TO INSECTS[1]

ALBERT HARTZELL AND FRANK WILCOXON

The problem of the relative toxicity of pyrethrins I and II is one that up to the present time has not been satisfactorily settled. The importance of this question has been recently emphasized by Tattersfield (12). The original view of Staudinger and Ruzicka (11) was that pyrethrin II was slightly less toxic to insects than pyrethrin I. Tattersfield, Hobson, and Gimingham (13) working with more precise toxicological methods, concluded that pyrethrin I was about ten times as toxic as pyrethrin II to *Aphis rumicis* L. On the other hand Gnadinger and Corl (1, 2), working with kerosene extracts on house flies (*Musca domestica* L.) confirmed the early findings of Staudinger and Ruzicka that pyrethrin II was only slightly lower in toxicity than pyrethrin I. The present authors, in a previous publication (15), have presented evidence that pyrethrin I is considerably more toxic than pyrethrin II to *Aphis rumicis*. More recently Ripert and Gaudin (9) found that pyrethrin I was about two and one-half times as toxic as pyrethrin II to house flies, when tested by a method similar to that described recently by Nelson, Buc, Sankowsky, and Jernakoff (7).

It occurred to us that discrepancies in the findings of various investigators might be due either to different susceptibilities in the insects used or differences in the physical state of the pyrethrins at the moment of application. The present paper describes experiments designed to test this hypothesis.

PREPARATION OF SAMPLES VARYING IN RATIO OF PYRETHRINS I TO II

The material used was a commercial petroleum ether extract of pyrethrin resins containing 10.8 per cent of pyrethrin I and 13.58 per cent of pyrethrin II by the Seil method of analysis. The sample, after removing traces of solvent under vacuum, was dissolved in 365 cc. of acetic acid to which were added 365 cc. additional acetic acid with 75 cc. of water. The waxy material which settled out was filtered off with suction. The acetic acid solution was divided into three portions and was extracted four times with low boiling petroleum ether, a total of 300 cc. being used in each extraction. The further preliminary purification followed closely the procedure outlined by Haller and Acree (3). After the combined samples had been dissolved in aniline and agitated with potassium carbonate solution there were obtained 24 grams of a light yellow oil which gave on analysis

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 122.

26.4 per cent of pyrethrin I and 37.4 of pyrethrin II. This oil was then put through a four-stage four-row multiple extraction (5) as shown in Figure 1, using as solvent 75 cc. of petroleum ether and 50 cc. of acetic acid solution made by adding 100 cc. of water to 1000 cc. of glacial acetic acid. The four petroleum ether exit solutions were numbered consecutively from top to bottom, P.E.$_1$, P.E.$_2$, P.E.$_3$, and P.E.$_4$, while the four acetic acid exit solutions were numbered consecutively from left to right A.C.$_1$, A.C.$_2$, A.C.$_3$, and A.C.$_4$. The petroleum ether exit fractions were washed several times to remove the acetic acid, dried over anhydrous sodium sulphate and the solvent removed *in vacuo*.

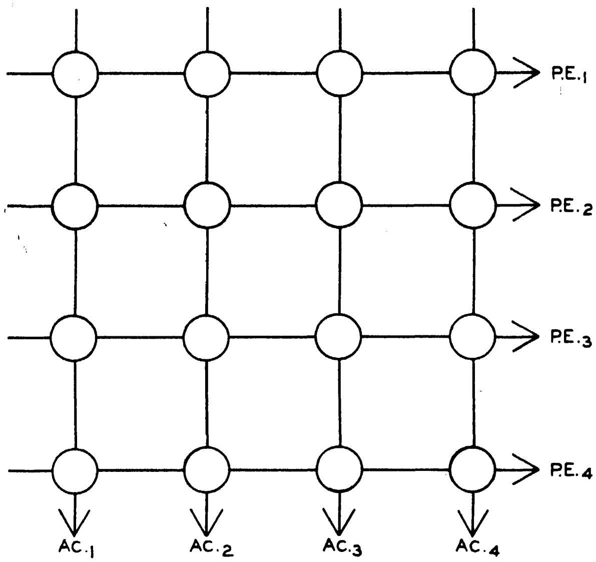


FIGURE 1. Multiple extraction scheme for purification of pyrethrins by use of immiscible solvents. The circles represent separatory funnels and the arrows indicate the direction of flow of the solvents used. P.E. refers to petroleum ether, A.C. to acetic acid.

The acetic acid fractions were poured into a large volume of water and the diluted emulsions extracted several times with petroleum ether. These petroleum ether extracts were washed free of acetic acid, dried over anhydrous sodium sulphate, and the solvent removed *in vacuo*. Thus eight fractions were obtained with the following weights and compositions by the Seil method (10) of analysis:

P.E.$_1$, 2.56 g., 17.96 per cent I, 5.86 per cent II; P.E.$_2$, 2.84 g., 35.7 per cent I, 8.92 per cent II; P.E.$_3$, 2.65 g., 42.5 per cent I, 19.8 per cent II; P.E.$_4$, 1.72 g., 47.75 per cent I, 27.7 per cent II; A.C.$_1$, 1.41 g., 3.4 per cent I, 72.2 per cent II; A.C.$_2$, 3.09 g., 7.7 per cent I, 74.2 per cent II; A.C.$_3$, 2.66 g., 16.27 per cent I, 63.0 per cent II; A.C.$_4$, 2.18 g., 29.3 per cent I, 52.2 per cent II.

In some cases pyrethrin II was also determined by the methoxyl method of Haller and Acree (3). Thus P.E.$_2$ contained 7.99 per cent of pyrethrin II while A.C.$_1$ contained 67.8 per cent pyrethrin II by this method. In general the methoxyl method gave results lower than the Seil method. The ratio of pyrethrin I to II in these samples varies from 4 in the case of P.E.$_2$ to 0.047 in the case of A.C.$_1$. These fractions were used for the following toxicity experiments.

TOXICITY EXPERIMENTS

The pyrethrin extracts, the preparation of which is described above, were tested on *Aphis rumicis*, using a method described in a previous publication (4). It will be noted in Table I that when extracts high in pyrethrin

TABLE I

COMPARATIVE TOXICITY TO APHIS RUMICIS OF PYRETHRUM EXTRACTS VARYING IN RATIO OF I TO II

Solvent or spreader	Concn. applied, %	Pyrethrins in original concentrate, %			Ratio I/II	% dead, duplicate tests
		Seil method				
		Pyrethrin I	Pyrethrin II	Total		
1 cc. acetone per 100 cc.	0.1	51.3	18.2	69.5	2.82	85.3 90.6
		5.5	77.5	83.0	0.07	8.3 27.0
With 0.5% Penetrol	0.03	35.7	8.92	44.6	4.0	81.0 86.2
		3.4	72.2	75.6	0.047	79.0 79.7
Acetone as solvent	0.03	35.7	8.92	44.6	4.0	93.7 84.0
		3.4	72.2	75.6	0.047	51.3 56.5

I are compared with extracts high in pyrethrin II, using acetone as a solvent for the pyrethrins and diluting with water, the pyrethrin I extracts are considerably more toxic than extracts high in pyrethrin II. When a miscible oil such as Penetrol is used as solvent, however, this difference in toxicity tends to disappear. The results of Tattersfield and Martin (14) with saponin as a spreader show a marked difference in toxicity to *Aphis rumicis* between pyrethrins I and II. In our experiments with *Aphis rumicis* pyrethrins I and II could be shown to exhibit practically equal toxicity when Penetrol was used as a solvent, and widely differing toxicity when acetone was used as a solvent.

When similar extracts were tested on house flies by means of the Peet-Grady[2] method (6, 8) using a kerosene carrier ("Crystalite"), the toxicity

[2] The writers are indebted to S. W. Penick & Co., and John Powell & Co., Inc., New York, New York, for facilities for making tests by means of the Peet-Grady method.

of extracts high in pyrethrin II compared favorably with extracts high in pyrethrin I, as shown in Table II. The kerosene carrier when used alone gave a kill of only 5 per cent. From 123 to 1000 house flies were used in each test.

TABLE II

COMPARATIVE TOXICITY TO HOUSE FLIES OF PYRETHRUM EXTRACTS IN "CRYSTALITE" (PEET-GRADY METHOD)

Pyrethrins in original concentrate, %				Ratio I/II	Concn. applied, mg./100 cc.	% dead after 24 hours
Seil method			Pyrethrin II Haller method			
Pyrethrin I	Pyrethrin II	Total				
35.7	8.92	44.62	7.99	4.0	30	48.2
3.4	72.2	75.6	67.8	0.047		42.2
47.7	27.7	75.4	16.02	1.72	38	68.7
29.3	52.2	81.5	40.7	0.56		68.8

Comparative tests were made also on house flies by means of a modified method described by Nelson and others (7). Adult house flies four to seven days old were chilled for 30 minutes at −6° C., until they became quiescent. They were then removed to a cold room at 3° C. The solution was applied from a micropipette capable of delivering a drop of 0.75 cu. mm. The flies were lifted by means of the wing so that the ventral surface of the thorax touched the drop of liquid issuing from the pipette. After treatment the flies were placed in a 400 cc. beaker and removed to room temperature and this beaker was placed in a 2-l. beaker containing a wad of cotton saturated with milk. The 2-l. beaker was covered with cheesecloth and the flies allowed to recover and counts of living and dead were made after 24 hours. As the live flies left the smaller beaker to feed on the milk, a separation of living and dead flies was accomplished facilitating count-

TABLE III

COMPARATIVE TOXICITY TO HOUSE FLIES OF PYRETHRUM EXTRACTS (MODIFIED NELSON METHOD)

Pyrethrins in original concentrate, %				Ratio I/II	Concn. applied*	Age of flies, days	% dead
Seil method			Pyrethrin II Haller method				
Pyrethrin I	Pyrethrin II	Total					
35.7	8.92	44.62	7.99	4.0	30 mg./ 100 cc.	4	37.9
3.4	72.2	75.6	67.8	0.047			50.0
35.7	8.92	44.62	7.99	4.0	30 mg./ 100 cc.	7	90.5
3.4	72.2	75.6	67.8	0.047			78.0

* These solutions were diluted 1:10 with ethyl alcohol before application.

ing. Approximately 50 flies were used in each test. The results indicate (Table III) that the toxicity of extracts high in pyrethrin I compares favorably with extracts high in pyrethrin II. The differences obtained were not statistically significant considering the number of flies used. Flies tested four days after emergence as adults were considerably more resistant to the extracts than those that were tested seven days after emergence.

These results appear to explain the divergent views held by different investigators regarding the relative toxicity of pyrethrins I and II. It appears that even when the same insect is used the ratio of toxicities observed depends on the mode of application. It appears likely that the physical condition of the toxic agent as applied is a determining factor in the relative toxicity of the pyrethrins. In kerosene the pyrethrins are of course in solution. When aqueous sprays are used, made by pouring acetone solutions of the pyrethrins into water, the latter are thrown out of solution, forming emulsions of varying degrees of stability. No doubt the penetration into the insect is dependent on the physical state of the material as applied, and this accounts for the varying results reported by different investigators.

SUMMARY

A partial separation of pyrethrins I and II was effected by multiple extraction using as solvents petroleum ether and aqueous acetic acid. Extracts were obtained in which the ratio of I to II varied from 4 to 0.047 according to the Seil method of analysis.

The comparative toxicity of pyrethrum extracts varying in ratio of pyrethrins I and II was determined on *Aphis rumicis* using acetone and a miscible oil as solvents for the pyrethrins with water. When extracts high in pyrethrin I were compared with extracts high in pyrethrin II using acetone as a solvent, the pyrethrin I extracts were considerably more toxic than extracts high in pyrethrin II. When a miscible oil such as Penetrol was used as a solvent the difference in toxicity tended to disappear.

When similar extracts were tested on house flies (*Musca domestica*) by means of both the Peet-Grady method and a modified Nelson method the differences obtained in the toxicity of extracts high in pyrethrin I and extracts high in pyrethrin II were not statistically significant.

The results indicate that the physical condition of the pyrethrins at the time of application is a determining factor in the relative toxicity at least so far as *Aphis rumicis* and *Musca domestica* are concerned. The relative toxicity of pyrethrins I and II depends almost entirely upon the method of application used.

LITERATURE CITED

1. GNADINGER, C. B. Pyrethrum flowers. 269 pp. McLaughlin Gormley King Co., Minneapolis, Minn. 1933.

2. GNADINGER, C. B., and C. S. CORL. Studies on *Pyrethrum* flowers. I. The quantitative determination of the active principles. Jour. Amer. Chem. Soc. **51**: 3054–3064. 1929.
3. HALLER, H. L., and FRED ACREE, JR. Constituents of pyrethrum flowers. Determination of pyrethrin II. Indus. & Eng. Chem. Anal. Ed. **7**: 343–344. 1935.
4. HARTZELL, ALBERT, and FRANK WILCOXON. Some factors affecting the efficiency of contact insecticides. II. Chemical and toxicological studies of pyrethrum. Contrib. Boyce Thompson Inst. **4**: 107–117. 1932.
5. HUNTER, T. G., and A. W. NASH. Liquid-liquid extraction systems. Computations for solvent refining oils. Indus. & Eng. Chem. **27**: 836–845. 1935.
6. NELSON, FRANK C. Changes and additions to the Peet-Grady method. Soap **10**(8): 81–83, 85, 97, 99, 101, 103. 1934.
7. NELSON, F. C., H. E. BUC, N. A. SANKOWSKY, and M. A. JERNAKOFF. Evaluating liquid insecticides. A new method for evaluating the relative toxicity of a liquid insecticide. Soap **10**(10): 85, 87, 89, 91, 105, 107. 1934.
8. PEET, C. H., and A. G. GRADY. Studies of insecticidal activity I. Testing insecticides against flies. Jour. Econ. Ent. **21**: 612–617. 1928.
9. RIPERT, J., et O. GAUDIN. Mesure de la toxicité de la pyréthrine I et de la pyréthrine II. Ann. Fals. et Fraudes **29**: 132–141. 1936.
10. SEIL, HARVEY A. Estimation of pyrethrins. Soap **10**(5): 89, 91, 111. 1934.
11. STAUDINGER, H., and L. RUZICKA. Insect poisons. X. Synthesis of pyrethrins. Helvetica Chim. Acta **7**: 448–458. 1924. (*Abst. in* Chem. Abstr. **18**: 2137. 1924.)
12. TATTERSFIELD, F. The present status of pyrethrum and derris investigation. Soap **11**(7): 87–88, 113. 1935.
13. TATTERSFIELD, F., R. P. HOBSON, and C. T. GIMINGHAM. Pyrethrin I and II. Their insecticidal value and estimation in pyrethrum (*Chrysanthemum cinerariaefolium*). I. Jour. Agric. Sci. **19**: 266–296. 1929.
14. TATTERSFIELD, F., and J. T. MARTIN. The loss of activity of pyrethrum. II. Jour. Agric. Sci. **24**: 598–626. 1934.
15. WILCOXON, FRANK, and ALBERT HARTZELL. Some factors affecting the efficiency of contact insecticides. III. Further chemical and toxicological studies of pyrethrum. Contrib. Boyce Thompson Inst. **5**: 115–127. 1933.

HYDROGEN SULPHIDE INJURY TO PLANTS[1]

S. E. A. McCallan, Albert Hartzell, and Frank Wilcoxon

The effect of hydrogen sulphide on green plants appears to have received little attention (3), while sulphur dioxide because of its relation to industrial smoke injury has been the subject of considerable study (3, 4, 14). Several investigators (2, 6, 7, 8) have shown that when sulphur dust is applied to the leaves of plants an interaction takes place resulting in the production of hydrogen sulphide. Furthermore, it is well known that sulphur fungicides, particularly lime sulphur, frequently cause spray injury.

Accordingly, a brief study has been made of the toxic action of hydrogen sulphide gas on green plants, in which attention was directed to symptoms, species sensitivity, concentration of hydrogen sulphide, temperature, and similarity to lime sulphur injury.

MATERIALS AND METHODS

All tests were performed out-of-doors, during fair days from June to September. Fumigation chambers shaded by cheesecloth and equipped with glass sides, tops, and doors were employed. The hydrogen sulphide gas was obtained from commercial tanks and introduced into a current of air driven by a blower, the concentration being controlled by pressure readings on a small glass venturimeter. The mixture escaped through a small exit hole. From time to time samples were withdrawn from the chamber by means of a gas burette and analyzed for hydrogen sulphide by the method of Almy (1). Due to variations in temperature and wind pressure the average control of the concentration was only within 20 per cent. The duration of all tests was five hours through the middle portion of the day. Temperature and relative humidity within the chambers were recorded.

The following 29 species of plants were tested: apple (*Pyrus malus* L.), aster (*Callistephus chinensis* Nees.), kidney bean (*Phaseolus vulgaris* L.), buckwheat (*Fagopyrum esculentum* Moench.), calliopsis (*Coreopsis* sp.), carnation (*Dianthus caryophyllus* L.), castor bean (*Ricinus communis* L.), sweet cherry (*Prunus avium* L.), white sweet clover (*Melilotus alba* Desr.), coleus (*Coleus blumei* Benth.), cornflower (*Centaurea cyanus* L.), cosmos (*Cosmos* sp.), cucumber (*Cucumis sativus* L.), Boston fern (*Nephrolepsis exaltata* Schott var. *bostoniensis* Davenport), gladiolus (*Gladiolus* sp.), nasturtium (*Tropaeolum minus* L.), peach (*Prunus persica* [L.] Stokes), pepper (*Capsicum frutescens* L.), California poppy (*Eschscholzia californica* Chamb.), purslane (*Portulaca oleracea* L.), radish (*Raphanus sativus* L.),

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 120.

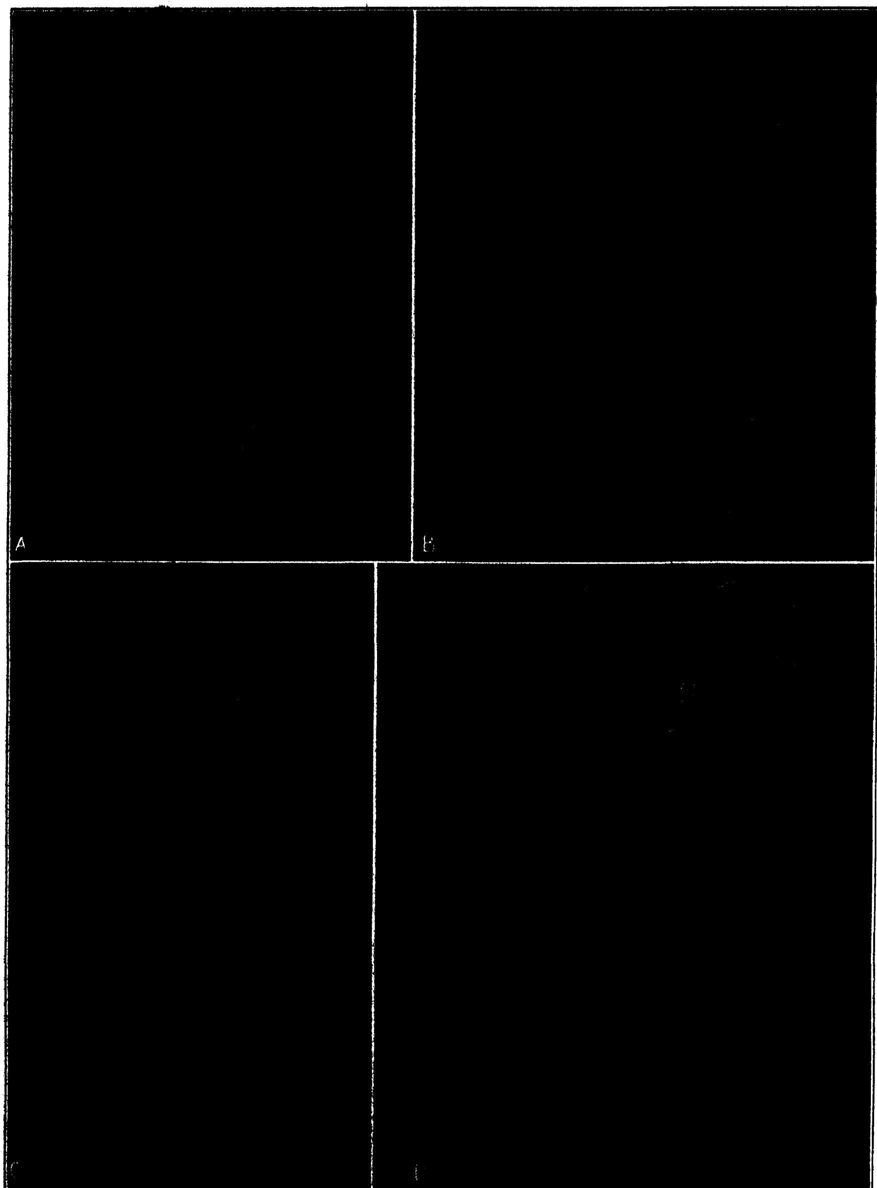


FIGURE 1. Symptoms of hydrogen sulphide injury. A. Aster. B. Salvia. C. Tomato. D. Soybean.

rose (*Rosa* sp.), salvia (*Salvia splendens* Ker.), soybean (*Glycine max* Merr.), strawberry (*Fragaria chiloensis* Duchesne), sunflower (*Helianthus debilis* Nutt.), tobacco (*Nicotiana glauca* R. Graham and *N. tabacum* var. Turkish), and tomato (*Lycopersicon esculentum* Mill.).

The plants were grown in the greenhouse in all cases except apple and cherry in which case small branches with the cut end immersed in water were used. In each experiment three potted plants of a given species were tested. In general there was but one plant per 4-inch pot except where the growth habit allowed more. Such plants were buckwheat, kidney bean, clover, calliopsis, nasturtium, pepper, poppy, purslane, and radish, where three to ten plants grew in a pot. All plants received the same treatment in the greenhouse and were watered about two hours before fumigation unless otherwise specified. At the end of the treatment the plants were removed from the fumigation chambers, left out-of-doors, and kept under observation for several weeks.

EXPERIMENTAL RESULTS

SYMPTOMS

Injury from hydrogen sulphide first appears on the young growing tissue and regions of most rapid elongation. Increases in severity of treatment will cause an extension of symptoms to the older tissue. The symptoms are necrotic, scorching being most typical. Yellowing may also occur and in some cases a curling and puckering may result due possibly to the continued growth of healthy tissue adjacent to necrotic areas.

Immediately on removal from the fumigation chamber, the injured areas exhibit wilting but within 24 hours have become necrotic. In most species there is no further change in appearance following the first day or two after treatment. A few plants, however, such as salvia, soybean, buckwheat, cucumber, and gladiolus may continue to show further evidence of injury. Occasionally, plants first recorded as severely injured may ultimately die. In general, however, unless the treatment has been very severe, growth is eventually resumed and the plant recovers.

A brief description of the symptoms expressed by species studied in detail follows:

Aster. Scorching of young inner involucre bracts, axillary buds, base of young leaves, and margins of next older leaves (Fig. 1 A).

Buckwheat. Death of young shoots, marginal scorching, and marginal upward rolling of younger leaves (Fig. 3 A, left).

Salvia. Scorching of crown and of shoots and young leaves in axils of old leaves (Fig. 1 B). Definite light brown necrotic areas at base of upper leaves (Fig. 2 B).

Soybean. Generally a delay in full expression of symptoms. Young shoots killed, marginal scorching of next older leaves (Fig. 1 D).

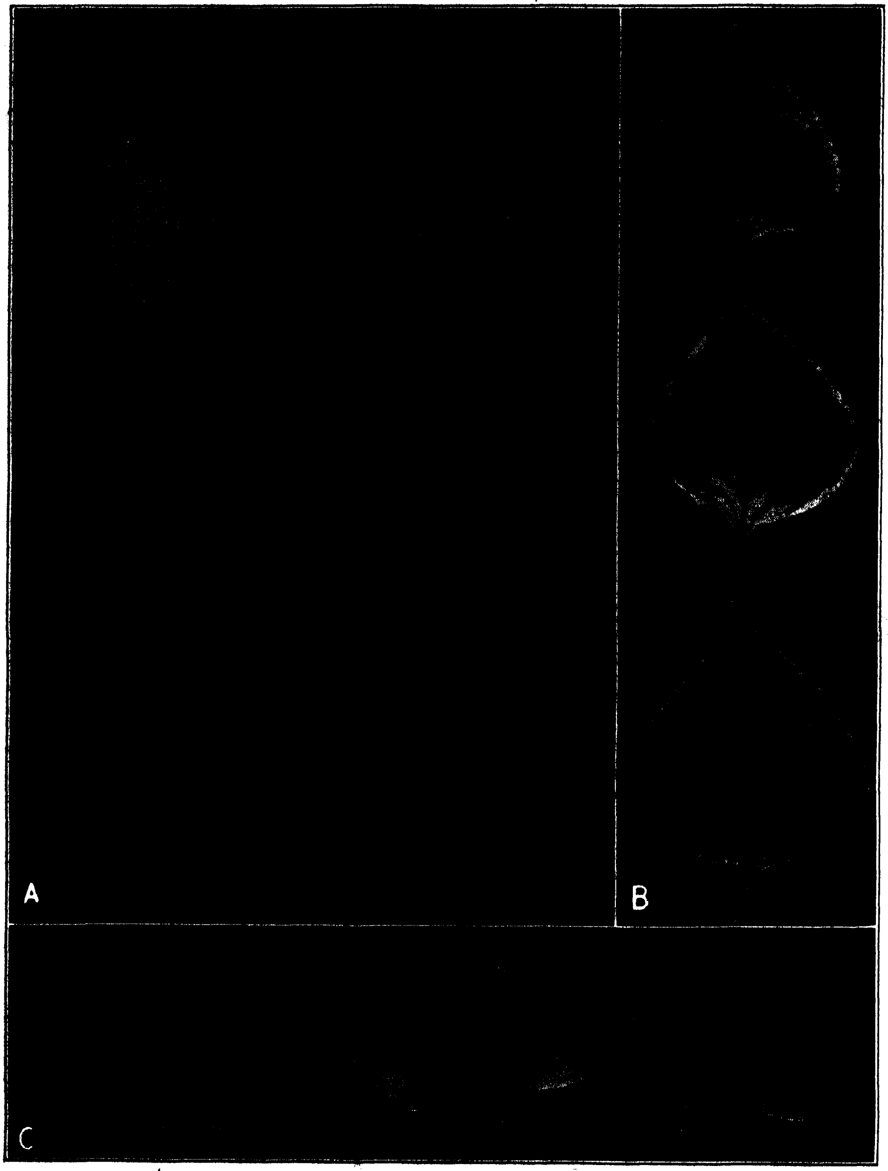


FIGURE 2. Hydrogen sulphide injury. A. Symptoms on sunflower. B. Basal scorching on upper leaves of salvia. C. Specific responses by different aged leaves of tobacco. All plants similar and treated alike.

Sunflower. Distinct interveinal, scorched zones, location of which is dependent on age of leaf. In general, youngest leaves scorched at tip, third or fourth pair of leaves scorched across center, and next pair scorched at base (Fig. 2 A). Older leaves generally unaffected though in some cases tip burning of oldest pair. More severe treatment causes in addition a severe scorching of crown, and puckering and contraction of leaves.

Tobacco. Crown and youngest leaves, especially at tip, show definite scorching. Interveinal, necrotic areas on basal and middle portion of intermediate leaves (Fig. 2 C).

Tomato. Scorching of terminal shoot and of leaflets and midrib area at base of younger leaves (Fig. 1 C).

Comparison with Sulphur Dioxide Injury

According to Zimmerman and Crocker (14) sulphur dioxide injury is characterized in dicotyledonous leaves by dead interveinal areas, the middle-aged leaves being the most sensitive and the young leaves least sensitive. Thus it is seen that the symptoms of hydrogen sulphide injury are distinctly different from those of sulphur dioxide.

EFFECT OF CONCENTRATION

Since the concentration of hydrogen sulphide was not controlled to a fine degree and since it was impossible to control the temperature, the experimental results have been arranged in groups according to the mean concentration per test. The mean temperature for each group, by this arrangement, did not differ significantly. The results are shown in Table I. It will be seen that there is a great variation in species sensitivity, and that over the sensitive range there is a uniformly increasing response to increase in concentration. Hydrogen sulphide gas is much less toxic than sulphur dioxide since the latter will cause injury at concentrations in the vicinity of 1 p.p.m. (14).

EFFECT OF TEMPERATURE

The effect of temperature is not apparent in Table I because the mean temperatures of each group were approximately the same. If the individual experiments are examined, however, it will be found that temperature is a factor of importance at least equal to that of concentration. This influence may be seen in Table II, where the results of experiments at high concentrations but low temperatures may be compared with those at low concentrations and high temperatures. In general more injury resulted at the higher temperatures despite the fact that the concentrations were lower.

TURGOR IN RELATION TO INJURY

It has been shown by Zimmerman and Crocker (14) that wilted plants are more resistant to sulphur dioxide than are turgid ones. Presumably

TABLE I

HYDROGEN SULPHIDE INJURY* AT DIFFERENT CONCENTRATIONS; MEAN TEMPERATURE 74°–81° F.; RELATIVE HUMIDITY 82–100 PER CENT; DURATION 5 HOURS

Plant species	Total plants	Concentration H_2S in p.p.m. 20–40	40–50	50–60	60–80	200–400
Carnation	12	o	o	—	—	o
Purslane	15	—	—	o	—	—
Boston fern	3	—	—	o	—	—
Apple	3	—	—	o	—	—
Cherry	3	—	—	o	—	—
Strawberry	9	—	—	o	—	o
Peach	24	o	o	—	x	o
Coleus	27	o	o	—	x	x
Pepper	18	—	—	x	—	xx
Rose	6	—	—	xx	—	xx
Castor bean	3	—	—	xx	—	—
Gladiolus	15	o	x	—	o	xxx
Sunflower	27	o	x	xx	xxx	xxx
Nasturtium	20	—	xx	—	—	—
Buckwheat	115	xx	xx	xx	xxx	xxx
Cornflower	12	o	xxx	—	xxx	xxx
Turkish tobacco	24	x	xx	xx	xxx	xxxx
Aster	30	xx	xx	xx	xxx	xxxx
Soybean	140	o	xx	xxx	xxx	xxxx
Kidney bean	12	—	—	xxx	—	—
Tobacco (*N. glauca*)	3	—	—	xxx	—	—
Cucumber	15	xx	—	xxx	xxx	xxxx
Salvia	24	x	xx	xxxx	xxxx	xxxx
Poppy	16	—	—	xxxx	—	—
Tomato	40	xx	xxx	xxx	xxxx	xxxx
Clover	30	—	xxx	—	—	—
Radish	120	xx	xxx	—	xxxx	xxxxx
Calliopsis	12	—	xxxx	—	—	—
Cosmos	12	—	xxxxx	—	—	—

* o = none, x = very slight, xx = slight, xxx = moderate, xxxx = severe, xxxxx = very severe.

TABLE II

EFFECT OF TEMPERATURE ON HYDROGEN SULPHIDE INJURY*

Temperature ° F.	76	80	90
Concentration p.p.m.	390	215	72
Peach	o	o	xx
Sunflower	o	xxx	—
Buckwheat	xx	xx	xxx
Cucumber	—	xxxxx	xxx
Soybean	xxxx	—	xxx
Aster	xxxx	—	xxxx
Cornflower	—	xxx	xxxxx
Tomato	xxx	—	xxxxx
Salvia	xxxx	—	xxxxx

* See footnote Table I.

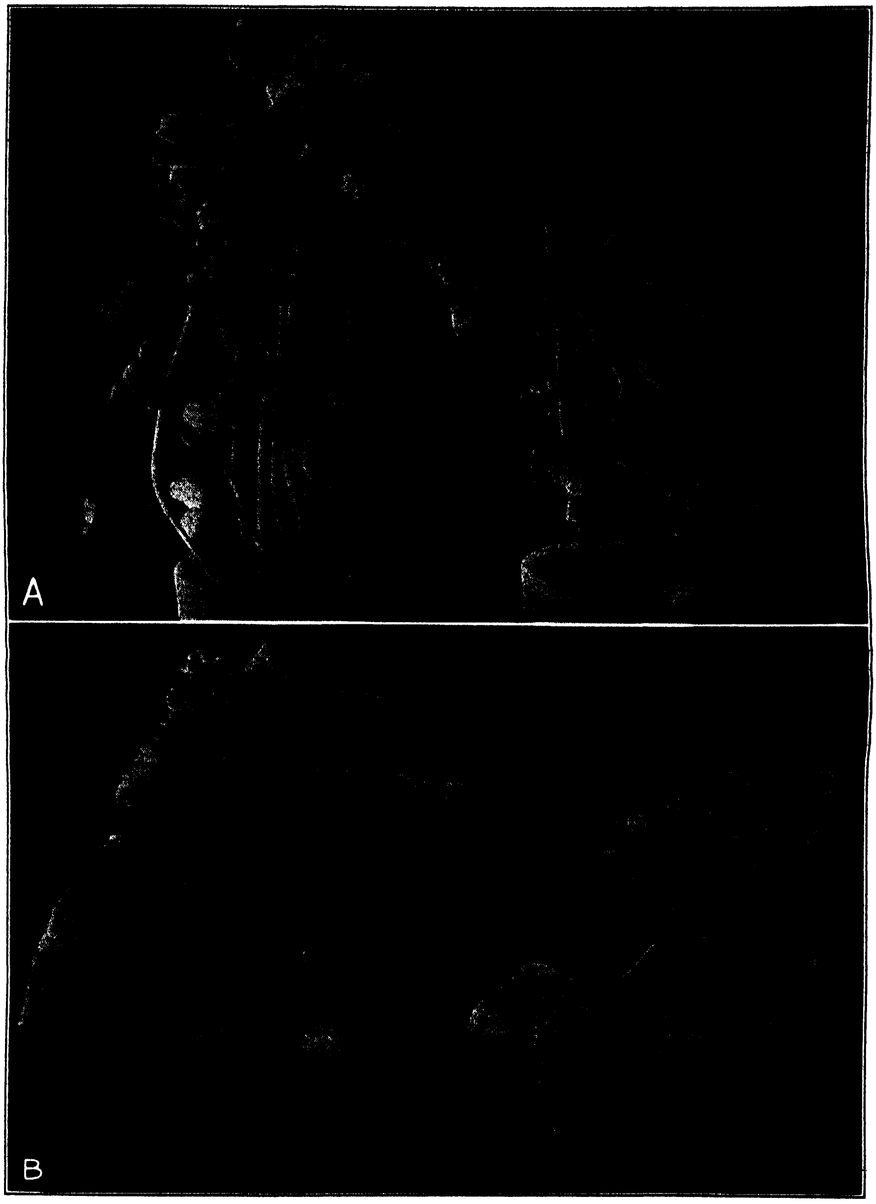


FIGURE 3. Comparison of hydrogen sulphide injury with lime sulphur injury. Hydrogen sulphide treated plants on left, lime sulphur on right. A. Buckwheat. B. Sunflower. Photographed 6 days after treatment (12 days in case of H_2S sunflower) and evidence of growth and recovery may be seen especially on sunflower. The spotting on lime sulphured plants is spray residue.

closing of the stomata due to wilting has prevented the entrance of sulphur dioxide.

In an experiment to test this effect on hydrogen sulphide injury, two plants each of aster, buckwheat, sunflower, and tomato remained without watering for 44 hours, at which time they showed apparent wilting. Control plants were watered as usual. Both lots of plants were then subjected to five hours of hydrogen sulphide fumigation, at a concentration of approximately 60 p.p.m. and temperature of 75° F.

No difference in injury was observed for the aster and sunflower, all plants showing very slight injury. However, while the turgid buckwheat and tomato plants showed respectively slight and moderate injury the wilted plants exhibited no symptoms of injury.

COMPARISON WITH LIME SULPHUR INJURY

Plants of aster, coleus, buckwheat, sunflower, tobacco, and tomato were sprayed with lime sulphur 1–20. After two days all plants showed moderate injury except those of coleus and tobacco which remained healthy. The type of injury produced by the lime sulphur appeared identical with that, previously described in detail, for hydrogen sulphide. Comparisons of injury produced by hydrogen sulphide and by lime sulphur are shown in Figure 3.

Lime sulphur injury has been attributed to various factors (5, 9, 10, 11, 12, 13). Wallace early pointed out that the action of Bordeaux mixture and lime sulphur was different in that the injury produced by the latter occurred within a few days (12). Krout believed that high temperature and high humidity would result in injury (5). It is perhaps significant that these factors are also important in hydrogen sulphide injury.

SUMMARY

1. The effect of hydrogen sulphide gas on 29 different species of green plants was studied. The plants were fumigated in glass chambers out-of doors during the growing season, the compressed hydrogen sulphide gas being introduced into an air stream which was blown through the chamber.

2. Young rapidly elongating tissue is characteristically more sensitive to hydrogen sulphide injury than older tissue. Typical symptoms are a scorching of the young shoots and leaves, basal and marginal scorching of next older leaves, and mature leaves unaffected. Symptoms are usually fully expressed within a few days of treatment.

3. The different species varied widely in their response: carnation, purslane, Boston fern, apple, cherry, peach, strawberry, and coleus showed no appreciable injury at concentrations below 400 p.p.m.; pepper, rose, nasturtium, castor bean, gladiolus, sunflower, buckwheat, and cornflower, slight to moderate injury at concentrations from 40 to 400 p.p.m.; and

soybean, Turkish tobacco, aster, kidney bean, cucumber, tobacco (*N. glauca*), salvia, poppy, tomato, clover, radish, calliopsis, and cosmos, slight injury below 40 p.p.m. and severe injury and death above 400 p.p.m.

4. Temperature is as important as concentration, injury increasing rapidly with increases in temperature.

5. In some cases wilted plants appear less sensitive to hydrogen sulphide injury than normal turgid plants.

6. Plants tested for lime sulphur injury (aster, buckwheat, sunflower, and tomato) showed symptoms identical with those produced by hydrogen sulphide.

LITERATURE CITED

1. ALMY, L. H. A method for the estimation of hydrogen sulfide in proteinaceous food products. Jour. Amer. Chem. Soc. **47**: 1381–1390. 1925.
2. BARKER, B. T. P. Investigations on the fungicidal action of sulphur: IV. Third progress report. Long Ashton Agric. & Hort. Res. Sta. Ann. Rept. **1929**: 130–148. 1929.
3. HASELHOFF, E., G. BREDEMANN, und W. HASELHOFF. Entstehung, Erkennung und Beurteilung von Rauchschäden. 472 pp. Gebrüder Borntraeger, Berlin. 1932.
4. HOLMES, J. A., EDWARD C. FRANKLIN, and RALPH A. GOULD. Report of the Selby Smelter Commission. U. S. Bur. Mines Bull. 98. 528 pp. 1915.
5. KROUT, WEBSTER S. Combating apple scab. Spraying and dusting experiments in 1922. Massachusetts Agric. Exp. Sta. Bull. 214: 29–41. 1923.
6. LIMING, O. NEAL, and H. C. YOUNG. Toxicity of sulphur to spores of *Sclerotinia cinerea* as affected by the presence of pentathionic and other sulphur acids. Jour. Agric. Res. **40**: 951–962. 1930.
7. McCALLAN, S. E. A., and FRANK WILCOXON. The fungicidal action of sulphur. II. The production of hydrogen sulphide by sulphured leaves and spores and its toxicity to spores. Contrib. Boyce Thompson Inst. **3**: 13–38. 1931.
8. MARSH, R. W. Investigations on the fungicidal action of sulphur. III. Studies on the toxicity of sulphuretted hydrogen and on the interaction of sulphur with fungi. Jour. Pomol. & Hort. Sci. **7**: 237–250. 1929.
9. MARTIN, HUBERT. The defoliation of gooseberries by sulphur-containing sprays. Jour. South East. Agric. Coll. Wye **27**: 182–185. 1930.
10. SAFRO, V. I. An investigation of lime-sulfur injury its causes and prevention. Oregon Agric. Exp. Sta. Res. Bull. 2. 32 pp. 1913.
11. SHOEMAKER, J. S. Lime sulphur injury. Sci. Agric. **4**: 180–184. 1924.
12. WALLACE, ERRETT. Spray injury induced by lime-sulphur preparations. New York [Cornell] Agric. Exp. Sta. Bull. 288: 101–137. 1910.
13. YOUNG, H. C., and R. C. WALTON. Spray injury to apple. Phytopath. **15**: 405–416. 1925.
14. ZIMMERMAN, P. W., and WILLIAM CROCKER. Toxicity of air containing sulphur dioxide gas. Contrib. Boyce Thompson Inst. **6**: 455–470. 1934.

AN IMPROVED METHOD FOR ASHING OF PLANT MATERIAL

W. D. STEWART AND JOHN M. ARTHUR

INTRODUCTION

In a recent study of the effect of light and other external factors on the ash constituents of plants a method of ashing plant material was developed which conserves the more volatile mineral elements and at the same time possesses good reproducibility and admits of more accurate duplication. The official method of the A. O. A. C. (2, p. 278) for the ashing of grain and stock feeds was found to be unsatisfactory. Poor duplication invariably resulted from attempting to follow the vague directions given in this procedure. In the Handbuch der Pflanzenanalyse (9) two methods of ash analyses are described. One is similar to the official methods and another consists of ashing the plant material with the addition of water, alcohol, ammonium nitrate, ammonium carbonate, or oxalic acid over a bunsen burner. These methods were found to have two major sources of error; range of temperature recommended and poorly defined procedure. A dull red heat permits a range of temperature from 550° to 650° C. (6), and the time of heating is never accurately specified. The direction "heat to a constant weight" ignores the fact that some ash constituents undergo volatilization with continued heating. This loss is very marked with material yielding an acid ash. Recognition of the losses by volatilization when these methods were used has led to the introduction of the bomb, or to the wet ashing process and similar technique.

Since ash content is an excellent index for quality of flour, the cereal chemists have studied the effects of various treatments and conditions for ashing flour and have improved the technique. They have used oxygen (5, 16) for hastening the oxidation, shortened the time of heating, and added known amounts of base (flour yields an acid ash) to fix the acidic constituents and reduce loss by volatilization.

Another concept known as the "total base" has been proposed and used for animal tissues and fluids. The material is ashed with sulphuric acid. It is assumed that the bases are converted into sulphates and the sulphate is determined and deducted from the weight of ash to give total base. Some workers, finding the variation between duplicates smaller, have ashed plant material with sulphuric acid and used this instead of the value from the usual method for an appraisal of mineral content.

The old procedures for ashing should be modified to give a reproducible method by which a reliable measure of the mineral content may be obtained. All details of the procedure should be defined exactly so as to have a standard basis for computation. The material should be dried in weighing

bottles at some given temperature for a stated period, allowed to cool in a desiccator over a given desiccant, weighed out by difference into tared ashing capsules of standard size and material, as the hygroscopic materials present absorb moisture rapidly and in varying amounts depending upon humidity. The capsules should be placed in an oven of uniform, definite temperature, at the lowest temperature consistent with removal of carbon in the presence of a stream of O_2, and heated for a determined period of time, removed, cooled and weighed.

A method of ashing is proposed and a comparison is made of results with this method and with those of the usual method of ashing in an open muffle.

MATERIAL AND METHODS

Ashing procedure. Only tissue from plants protected from spattering of soil by rain or watering was used. The air-dry material was ground in a "Nixtamal" mill and mixed. Any particles of metal loosened from the mill were removed from the material with a small magnet. Small weighing bottles were filled loosely with the sample, placed in an oven at 100° C. for two hours, removed and allowed to cool in a desiccator over calcium chloride. Portions were then weighed into small porcelain capsules that had been acid treated and cleaned well previously. Ashing was carried out in both muffle and tube furnaces. The muffle was a Hoskins M. U. Electric, Type 60, with rheostat adjusted to give an operating temperature of approximately 650° C. as determined by a commercial thermoelectric pyrometer. The tube furnace was an electric multiple unit furnace, Type 77, made by the Hevi-duty Electric Company, and furnished with resistance units to obtain a temperature of 450° C. Its bore was sufficient to take a tube 30 mm. in diameter. In later work a tube furnace was used similar to this except larger with a bore sufficient to take a 65-mm. tube. The capsules used in the muffle furnace were of the round type, 47 mm. in diameter by 11 mm. high, while those used in the tube furnace were of the long shallow type, approximately 90 mm. long by 13 mm. wide by 9 mm. high. Smaller capsules were used for smaller samples. All of the larger sample portions were weighed by difference in weight of bottle before and after removal of material into the tared porcelain capsules. The capsules were flamed with a match before being placed in the furnaces for ashing since some tissues are of such composition as to ignite explosively upon heating. Capsules were placed in the hot furnace for the required period, removed, permitted to cool in desiccators over calcium chloride, and weighed. For ashing in the tube furnace, capsules were placed in a perforated Monel Metal carrier which was inserted into a pyrex or silica combustion tube. The tube was then put into the hot furnace. The capsules and carrier were manipulated so that the capsules were all equidistant

from the center of the tube and of the furnace. Air was pulled over the samples for 15 minutes; after this interval dry, washed oxygen was led over the samples through a bubble counter at the rate of approximately 60 bubbles per minute until the end of the combustion period. The capsules were then removed, cooled and weighed as with those from the muffle. Other samples were treated with sulphuric acid and ashed for eight hours in the muffle at 650° C. Sulphate was determined and this value subtracted from the weight of ash, the difference being the "total base."

Analytical methods. Gravimetric methods were used for estimating sulphur and chlorine. The reverse method of precipitation of sulphate as barium sulphate and of chlorine as silver chloride described by Popoff and Neuman (15) was followed. Phosphorus was precipitated as ammonium phosphomolybdate and the usual volumetric procedure for its estimation applied. After removal of phosphorus by ashing with sulphuric acid and ferric sulphate (7) potassium was precipitated as the cobaltinitrite and estimated according to the procedure of Kramer and Tisdall (10). In addition both the perchlorate and chloroplatinate methods were used. Tissues were also oxidized by Neuman's wet ashing procedure and in a Parr bomb with sodium peroxide. Sulphate and chloride determinations from these ashings were used as the "standard values" for comparison with those obtained from the combustions in the tube and muffle furnaces.

RESULTS

ASHING IN MUFFLE AT 650° C. COMPARED WITH ASHING IN TUBE AT 450° C. WITH OXYGEN

Variation between duplicates and variation with repetition of process. Excellent cause for dissatisfaction with ashing in the muffle furnace has been the variation between duplicates and inability to duplicate values upon repetition of the process. From Coleman and Christie's observations (6) this might be attributed to unequal heating. They advise testing the furnace for "hot spots" and disuse of the space one inch from the rear and three inches from the front of the muffle for ashing.

Table I shows the results secured by ashing several types of plant material for 16 hours in the tube at 450° C. with oxygen and in the muffle at 650° C. A small number of capsules were placed in the muffle at one time near the center, so that the variation might be small. Yet the variation between duplicates was greater than that found with ashing in the tube, and the variation with repetition so great that comparable values were not to be had; whereas in the tube good agreement was obtained. A consistently higher ash was obtained at the lower temperature. Similar examples may be noted in several of the tables that follow.

TABLE I

COMPARISON OF VARIATION BETWEEN DUPLICATES AND VARIATION WITH REPETITION OF ASHING PROCESS. SAMPLES ASHED IN TUBE AT 450° C. WITH OXYGEN AND IN MUFFLE AT 650° C.

Material	Trial No.	% Ash, tube	% Ash, muffle
Buckwheat leaves	1	16.14 16.19 16.13 16.06	13.27 13.35 13.49 13.60
	2	16.15 16.35 16.16 16.24	14.00 14.48 13.80 14.10
Cotton leaves	1	15.42 15.77 15.49 15.80	13.00 12.30 12.96 12.82
	2	15.89 15.73 15.60	12.16 11.60 11.70
Tomato, entire plant. Sample 1	1	20.28 20.19 20.21	18.00 17.50 18.20
	2	20.16 20.30 20.35	17.00 17.50 17.60
Tomato, entire plant. Sample 2	1	21.22 21.59 21.27 21.57	
	2	21.59 21.72 21.48 21.36	

Effect of temperature and time of heating on ash content. Buckwheat leaves were ashed in the tube with oxygen at 450° C. and in the muffle at 650° C. for 8- and 16-hour periods. The data in Table II reveal no significant difference for the longer period of heating in either tube or muffle for this particular tissue. The results also furnish more evidence of the range of the variation between duplicates and between separate runs in both tube and muffle. Using a different sample of buckwheat leaves the ash content was found to be 18.04 per cent for the 8-hour period and 17.09 per cent for the 16-hour period in the tube, and 16.50 and 15.90 per cent for these periods in the muffle; values for cotton leaves were 20.00 and 19.20 per cent for the tube, and 18.40 and 18.00 per cent for the muffle. Tomato plants and tobacco leaves showed similar decreases with the longer period of heating. Apparently some samples show lower ash content with the longer period of heating and others do not.

TABLE II

EFFECT OF TIME OF HEATING ON ASH CONTENT OF BUCKWHEAT LEAVES; VARIATIONS WITH DUPLICATES AND WITH REPETITION OF PROCESS

Trial No.	% Ash with 8-hr. period		% Ash with 16-hr. period	
	Tube	Muffle	Tube	Muffle
1	17.15 16.81 17.37 17.37	14.60 14.22 14.00 14.54	16.66 16.23 16.78 16.53	14.68 14.06 14.90 14.07
	Av. 17.17	Av. 14.34	Av. 16.55	Av. 14.42
2	16.89 16.90 17.30 16.54	16.30 16.15 15.97 15.79	16.96 17.09 16.63 17.12	15.19 15.32 14.92 15.40
	Av. 16.90	Av. 16.05	Av. 16.95	Av. 15.20

It was reported (1) that the ash of flour when starting with a cold muffle or at a temperature of 200° C. was very black and the results high whereas the ash was of good appearance and the results lower if the process was started at 550° C. Tomato plant tissue placed in the cool tube and ashed for 16 hours gave a value of 20.22 per cent as compared with a value of 19.77 per cent when started in the hot tube (450° C.) and ashed for the same period. A different sample of tomato yielded 22.07 per cent ash for the cool tube and 21.59 per cent for the hot tube. Ashes from the samples placed in the hot tube were seemingly free of carbon but that of material started in the cold tube was not.

Size of sample and ash content. Six years of experience with ashing in the muffle, and two years with the tube at the temperatures mentioned have revealed no indication of a correlation between size of sample and ash content of leaves, stems or roots of such plants as tobacco, tomato, buckwheat, cotton, and corn. Over a thousand determinations have been made with samples ranging from 0.1 to 1.2 grams in the porcelain combustion boats, and from 0.8 to 2.5 grams in the circular types of ashing capsules. With the combustion boats the size of the boat increased with the amount of the sample so that no great variation in surface per unit of weight was had; with the larger capsules the range in weight was not great. Poor duplication might result, however, if samples differing for example as widely as 0.05 gram and 2.5 grams of dry material were placed in the larger capsules and ashed for comparison. Such a test was not made.

EFFECT OF TEMPERATURE AND TIME OF HEATING UPON VOLATILIZATION OF ASH

Since ash content was much higher at 450° C. than at 650° C. and since both appeared equally free of carbon, the nature of the difference was sought. Analyses for calcium, phosphorus, potassium, sulphur, and chlo-

rine of buckwheat leaves ashed in the tube and in the muffle were made. These data constitute Table III. Calcium and phosphorus contents were unaffected but chlorine and sulphur were less at the higher temperature. Analysis of the volatilized material from 50 grams of the buckwheat leaves ashed in the tube and collected in an absorption train of 5 per cent Na_2CO_3 gave values of 0.07 per cent for chlorine and 0.13 per cent for the sulphate computed as per cent of dry weight. With the use of the cobaltinitrite method a very noticeable drop in potassium occurred at the higher temperature. Later experience indicated that the cobaltinitrite method was untrustworthy; this should invalidate the size of the difference. Unfortunately insufficient material was available for an analysis by a better method.

TABLE III

ASHING IN TUBES AT 450° C. WITH O_2 FOR 16 HOURS VERSUS ASHING IN MUFFLE AT 650° C. FOR 16 HOURS; COMPOSITION OF BUCKWHEAT LEAVES

Method of ashing	% Ash	% Ca	% P_2O_5	% K (cobaltinitrite)	% SO_4	% Cl
Tube	19.37	2.95	1.11	4.75	0.94	1.12
Muffle	14.78	2.96	1.19	3.11	0.74	1.00

TABLE IV

ASHING OF BUCKWHEAT LEAVES IN TUBES AT 450° C. WITH O_2 VERSUS ASHING IN MUFFLE AT 650° C.; VOLATILIZATION OF ASH CONSTITUENTS

Method of ashing	% Ash	% CO_3	% Cl	% SO_4		% P_2O_5		% K (chloroplatinate)		% K (cobaltinitrite)	
	16 hrs.	16 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.
Tube	16.37 16.14 16.00 16.26 Av. 16.19	4.16	0.27	0.61	0.59	1.34	1.32	3.94	3.96	4.04	3.67
Muffle	14.11 13.64 13.87 13.73 Av. 13.83	2.42	0.08	0.63	0.65	1.33	1.32	3.86	3.87	3.58	3.23
Wet ash ($HNO_3+H_2O_2$)			0.57	0.65							
Parr bomb (Na_2O_2)			0.62					4.01*		6.27	

* K as perchlorate.

Another sample of buckwheat leaves, grown under conditions differing from the sample above, was ashed in the tube and muffle for both 8- and 16-hour periods and a more complete analysis of the ash made. The results of this experiment are shown in Table IV. Higher carbonate content accounts for most of the increase of ash in the tube. Loss of chlorine occurred in both tube and muffle but the loss was greater with combustion at the higher temperature. No significant loss of sulphur took place at either temperature.

TABLE V

ASHING OF TOBACCO IN TUBE WITH O_2 VERSUS ASHING IN MUFFLE; COMPARISON OF LOSSES OF MINERALS BY VOLATILIZATION

Method of ashing	% Ash		% CO_3		% Cl		% SO_4		% K (chloro-platinate)		% Na	
	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.	8 hrs.	16 hrs.
Tube 450° C.	23.50	23.30	5.02	4.71	2.24	2.20	2.36	2.42	6.40	6.51	2.52	2.58
Muffle 650° C.	21.12	21.11	3.68	2.80	2.21	2.16	2.32	2.41	6.25	6.26	2.50	2.55
Wet ash (HNO_3 + H_2O_2)					2.30		3.11					
Tube* 450° C.										6.27		
Muffle* 650° C.										6.31		
Muffle* 750° C.										2.68		

* Averages of 2 to 4 analyses on the same composite sample ashed at 450°, 650°, and 750° C. as indicated.

Dissimilarity of behavior of buckwheat leaves grown under different conditions led to the use of tobacco leaves for the same tests. The leaves were ashed as usual in the tube at 450° C. with oxygen and at 650° C. in the muffle for 8 and 16 hours. Data from this experiment are shown in Table V. Ash content with the tube was higher than that with the muffle, and the ash content dropped slightly for the longer period of heating at 450° C. but no change was evident for the 16-hour period at 650° C.; yet the carbonate content of the ash decreased with increased period of heating for both tube and muffle, and the decrease was greater at 650° C.

Chlorine values at 450° C. and at 650° C. did not differ appreciably and closely approached that secured with the wet ashing procedure; nor did the increased period of heating affect the values.

Sulphur content of this tissue was not altered by either the temperature of ashing or the period of heating but was less than that found by the wet ash process. The percentage of potassium was not changed by the longer period of heating but a loss was indicated for the higher temperature of ashing.

Sodium content was unaltered by either temperature or period of ashing.

TABLE VI

ADDITION OF CHEMICALS AS AIDS FOR ASHING IN THE TUBE AT 450° C.

Material	Treatment	% Ash	
		With	Without
Buckwheat—Leaves #1	Alcohol+glycerine*	19.30	18.77
Buckwheat—Leaves #2	Alcohol+glycerine*	20.31	19.67
Buckwheat—Stems #1	Alcohol+glycerine*	28.66	28.24
Buckwheat—Stems #2	Alcohol+glycerine*	26.14	25.94
Cotton—Leaves	Oxalic acid solution	16.00	15.48
Tobacco—Leaves #1	NH_4NO_3 solution	20.31	20.41
Tobacco—Leaves #2	HNO_3	20.96	20.41
Tomato—Leaves #1	H_2SO_4	16.00	12.50
Tomato—Leaves #2	H_2SO_4	18.41	14.00
Tomato—Stems #1	H_2SO_4	20.50	18.50
Tomato—Stems #2	H_2SO_4	18.60	15.70
Tomato—Roots #1	H_2SO_4	17.31	15.14
Tomato—Roots #2	H_2SO_4	16.80	14.20

* Equal parts of 95 per cent alcohol and glycerine.

Addition of chemicals as "aids for ashing." A mixture made up of equal parts of alcohol and glycerine is frequently added to material to be ashed to obtain a rapid and more complete removal of carbon. Sulphuric acid is also used. Sometimes nitric acid is added to an incompletely oxidized mass to aid in the decomposition of organic matter. Also, as stated in the introduction, followers of the German technique add ammonium nitrate or carbonate and oxalic acid to the material. These substances were added to material before ashing in the tube at 450° C. and the results compared with those secured from untreated material. The data in Table VI show the results of such addition; in most cases slight increases in ash resulted from such treatment, and for sulphuric acid the increase was large, often showing a difference as great as 4 per cent in the ashing of leaf tissue. The reasons for the large increase in ash resulting after sulphuric acid treatment are discussed in the following text.

Volatile compounds given off by material ashed in the muffle. Improvement of the ashing technique would be simplified if we were acquainted with the processes and conditions resulting in losses by volatilization. In-

formation as to the products condensing from the gases evolved by the material being ashed would be of value. In addition more accurate knowledge of the temperature of volatilization of various constituents known to be present in plant tissue is desirable.

In order to test briefly the first point regarding the volatile products arising from plant tissue, large quantities of buckwheat leaves were charred on a hot plate until the vapor evolved was invisible; they were then placed in the hot muffle at 650° C. A glass tube about one inch in diameter and two feet in length was fitted into the vent of the furnace, and the condensed material examined at intervals. At the end of 24 hours the tube was heavily clouded. The cloudiness formed two distinct bands, consisting of one region of the first two inches adjoining the furnace and a second band beginning approximately four inches from the furnace and extending the length of the tube. When observed under the microscope the tube was covered with crystals. The two fractions described were washed separately into flasks, concentrated, and microchemical tests made. Other portions were taken and allowed to crystallize in partial vacuo over calcium chloride. The material condensing at the lower temperature was isometric with a refractive index of 1.64. Microchemical tests showed ammonia and chloride as the chief constituents, with no sulphate. The crystals were identified as ammonium chloride. The fraction condensing at the higher temperature near the furnace was very rich in ammonia and sulphate but was contaminated with chlorine. In fact, ammonium chloride crystals were present on the same slide with the crystals which were identified as ammonium sulphate. Traces of potassium were present in the tube. Since tobacco leaves gave no evidence of chlorine loss the results of a similar test with this material are of interest. The leaves were placed in the muffle and condensation observed. No clouding of the tube occurred and no ammonium chloride crystals were present with use of quantities of material even greater than with buckwheat. Occasional cubes and plates were seen but insufficient amounts for identification.

To gain more specific knowledge of the volatilization temperatures of the salts commonly present in plant tissue, loss in weight tests were made on some of these constituents. The results are indicated in Table VII. "Reagent Quality" chemicals were used for the tests. The salts were dried at 115° C. for at least two hours before the first weighing was made. Temperature readings on the commercial thermoelectric pyrometer, used in determining muffle temperatures, were checked against a mercury thermometer reading to 550° C. The upper temperature ranges above 550° C. were assumed to be reasonably accurate. When potassium and calcium salts were heated to 450° C. they were found to lose from 0.22 per cent in the case of calcium carbonate to 3.03 per cent in the case of the oxide. These small losses are thought to be due to moisture and impurities since the

TABLE VII

AVERAGE LOSSES IN PER CENT FROM ANALYTICAL REAGENT QUALITY SALTS WHEN HEATED TO 450° C. AND ADDITIONAL LOSSES IN PER CENT WHEN THE SAME SAMPLES ARE HEATED FURTHER TO 650°, 700°, AND 750° C.

Salt used	450° C.	650° C.	700° C.	750° C.
	1 to 3 hours	8 hours	8 hours	8 hours
Potassium chloride	0.99	0.37	1.36	8.92
Potassium sulphate	1.11	0.33	0.00	0.00
Potassium carbonate	1.53	0.07	1.01	2.45
Calcium chloride	1.92	0.93	14.31	Melted
Calcium sulphate	1.37	0.40	0 00	0.00
Calcium carbonate	0.22	42.82*	—	—
Calcium oxide	3.03	0.55	0.00	0.00
Magnesium sulphate	32.61	0.33	—	0.52
250° C. for 16 hours	31.87			
Magnesium chloride	78.28	0.30	—	0.00
250° C. for 16 hours	74.72			

* Converted to calcium oxide.

losses with the exception of calcium carbonate are greatly decreased on the second heating at 650° C. Calcium carbonate has been converted almost completely to the oxide after the 8-hour period of heating at 650° C. with a loss in weight of approximately 43 per cent. There is some indication that even calcium chloride has been volatilized at 650° C. as the loss of 0.93 per cent is exceptionally high, while on the next heating for eight hours at 700° C. this salt loses over 14 per cent of its residual weight and has lost more than 17 per cent of its initial weight. Both potassium chloride and carbonate are beginning to volatilize or decompose at 700° C. with losses of 1.36 and 1.01 per cent respectively and after the period of eight hours at 750° C. these losses are increased to 8.92 and 2.45 per cent respectively. Magnesium sulphate shows an initial loss of 32.61 per cent at 450° C. Even after 16 hours at 250° C. this loss amounts to 31.87 per cent. This is believed due entirely to the water of crystallization since the salt shows very little further loss at 650° and 750° C. This was not found true of the chloride. Magnesium chloride decomposes at a low temperature forming first the oxychloride and then the oxide. At 450° C. it lost 78.28 per cent of its original weight. Analysis of the resulting salt after heating magnesium chloride at 450° C. for three and one-half hours showed only 0.94 per cent chlorides remaining. It is believed that the resulting mixture is practically all magnesium oxide which remains stable even when heated to 650° and 750° C. Analysis for magnesium after heating to 450° C. showed approximately 61 per cent magnesium, while the theoretical amount of magnesium in the compound magnesium oxide is 60.3+ per cent. When magnesium chloride was heated to only 250° C. for 16 hours it lost 74.7 per cent of its weight indicating that even at this low tempera-

ture it is very largely converted to the oxide. In contrast with the decomposition of magnesium chloride analysis of the potassium chloride remaining after heating to 750° C. for eight hours showed a potassium content of 52 per cent. The theoretical potassium content of the salt KCl is 52.4^{+} per cent. This indicates that the salt KCl sublimes as such at this temperature without decomposition, losing both potassium and chlorine. Magnesium chloride is converted to the oxide by the loss of chlorine but with no appreciable loss of magnesium even when heated to 750° C. Tests for both calcium and chlorine on the compound resulting when $CaCl_2$ is heated to slightly over 700° C. for six hours indicates that this salt sublimes as $CaCl_2$ similar to KCl but in addition decomposes to a slight extent (approximately 10 per cent) forming calcium oxide similar to the decomposition of magnesium chloride. Calcium chloride is, therefore, intermediate in this respect between potassium and magnesium chlorides.

It is evident from the above considerations of the salts of alkali metals that there should be little or no loss when the ashing temperature does not exceed 450° C. providing mixtures of these salts behave similarly to the individual salts. The only exception is magnesium chloride which would be found mainly as the oxide. Higher ashing temperatures would result in losses of both carbonates and chlorides as well as potassium and calcium. Since all of the sulphates tested are comparatively stable even at 750° C., plant tissues higher in sulphates and lower in chlorides and carbonates would give a higher ash. Also treatment of the tissue with sulphuric acid before ashing would give a higher ash, due to conversion of both chlorides and carbonates to sulphates. Such treatment has shown a consistent increase in the amount of ash of tomato, buckwheat, and other plant tissues of from 1 to 4 per cent, the equivalent, as a maximum figure, of a 25 per cent increase in the total weight of ash.

TOTAL BASE DETERMINATIONS

Total base determinations of blood and sera have been in use for several years as an aid for the diagnosis of certain pathological conditions. While the concept may be useful for the study of a few types of nutritional problems, it is in general rather limited in its application since interpretation is difficult. Theoretically the ashing with sulphuric acid should, upon volatilization of the acid radicals present, convert the bases to sulphates. Then determining the sulphate and subtracting this value from the weight of the ash would give a measure of the bases present.

Experiments were performed to test whether or not the acid radicals of the ash of plants were completely removed. Phosphorus is an essential element and is present in appreciable amounts so determinations were made for this constituent from tomato leaves ashed with and without the sulphuric acid. The comparisons may be had from Table VIII. The phos-

TABLE VIII

TOTAL BASE DETERMINATIONS OF TOMATO LEAVES

Sample No.	% Ash			% P_2O_5		% Total base
	Muffle	H_2SO_4 Muffle	Tube	Without H_2SO_4	With H_2SO_4	
1	12.97	18.63	14.40	1.45	1.35	9.03
2	11.05	16.20	12.41	1.66	1.63	7.92
3	12.25	18.60	13.75	1.68	1.55	8.78
4	11.65	17.09	12.88	1.52	1.44	7.98

phorus content was only slightly affected by the acid ashing so the total base percentages were probably high, varying with the amount of reduction in phosphorus.

DISCUSSION OF RESULTS

Oxygen has been used by the cereal chemist for the rapid ashing of flour, by the industrial chemist for the ashing of coal at high temperatures (8), and by the biochemist for the more modern technique for iodine analysis (13, 17, 18); but its application for the ashing of plant material at low temperatures has not been developed. The plant material used in these experiments ashed at 450° C. with oxygen, yielded almost without exception ash free of carbon. Ashing in the tube at 450° C. with oxygen at a pressure of one pound of oxygen is suggested as a probable improvement of technique permitting complete combustion of all types of material. This should be preceded by charring at 450° C. for 15 minutes without the pressure or in a dry, rapid stream of oxygen to avoid explosions. Brendel (5) used oxygen at a pressure of one pound in a muffle held at 1500° F. and obtained excellent combustion of flour within 45 minutes.

The decrease in variation between duplicates, and the small variation between values obtained with repetition of ashing in the tube compared with those secured in the muffle showed the former to be a more reliable method (Tables I and II). This is attributed to more uniform distribution of heat—since its heating units encircle the tube, to smaller variations in temperature and to the lower temperature of ashing. The Monel Metal carrier for the combustion capsules, being an excellent conductor of heat, aids in equalizing any local differences in temperature. Since the conditions are known and can be duplicated, the standardization so highly necessary to accuracy upon repetition is had.

Much has been written of the undesirable features of ashing at high temperature and of the advantages of a low temperature. Comparing the values obtained at 450° C. in the tube with those from the muffle at 650° C.

(Tables I, II, III, IV, and V) a higher ash content is found at the lower temperature. The temperature of ashing was found to be more important than the time of heating, but both may contribute to variation. The increase in ash at the lower temperature was found to consist of both higher carbonate content and higher content of volatile constituents especially chlorides, the increase in carbonate predominating. Decrease in ash with increased period of heating, when it occurred, was due almost entirely to decrease in carbonate content. Temperature of ashing was also the important factor affecting volatilization, increasing the time of heating from 8 to 16 hours, affecting the losses but slightly or not at all. Differences in amount and kind of constituents volatilized from different samples of the same species as well as from different species was observed. The sample of buckwheat used for the analyses in Table III showed a decrease in content of sulphur, chlorine, and potassium at the higher temperature, while the sample described in Table IV showed no loss of sulphur, but loss of chlorine, and slight loss of potassium at the higher temperature. Analyses of tobacco gave quite different results (Table V); loss of chlorine did not occur at either temperature, nor was the loss of sulphur any greater at 650° C. than at 450° C., and no loss of potassium occurred at 450° C. but a slight decrease in content at 650° C. Using a collecting train for the absorption of the elements volatilized from the sample of buckwheat described in Table III, proof was had of losses of chlorine and sulphur even at 450° C., though much less than the loss with ashing at 650° C.

Reduction in potassium content took place at the higher temperature of ashing (Tables III, IV, and V) regardless of the method used for its estimation. The decrease in potassium with the longer period of heating shown by the cobaltinitrite method (Table IV) should be disregarded since the chloroplatinate values indicated no change and the former method was found to be unreliable. Change in ratio of sodium to potassium has been shown to influence the composition of the cobaltinitrite precipitate (4, 19). The values obtained for potassium from buckwheat leaves ashed in a Parr bomb with sodium peroxide were 4.01 per cent as the perchlorate and 6.27 per cent as the cobaltinitrite (Table IV). This high value may be due to the high concentration of salts common to such fusions because of the large amount of sodium present. Preparation of solutions from sodium metal of approximately the same concentration as those found with fusions in the bomb, with no potassium present, gave precipitates when added to the cobaltinitrite following the usual procedure for the precipitation of potassium. Yet the content of potassium of the same tissue ashed in the tube gave values of 4.04 per cent as the cobaltinitrite which agreed closely with the perchlorate value from the bomb and with the chloroplatinate value 3.94 per cent from the tube. The cobaltinitrite method under certain

of the above conditions is shown to be unreliable and should not be used. Losses in potassium have been reported by Lockhart (12) and Bible (3) for fertilizer mixtures ashed by the official method, and the latter found that losses of phosphorus and potassium were prevented by addition of NaOH preceding ashing.

Lack of consistency as to elements volatilized with ashing of plant material must be associated with differences in composition—both organic and inorganic. The ratio of the basic elements and the presence or absence of certain elements that act as catalysts for reduction probably affect the degree of volatilization as well as the elements volatilized and the temperature at which such volatilization occurs. For example, Pierre (14) found that the usual losses of sulphur, chlorine, and phosphorus experienced with ashing of fertilizers by the official method could be avoided by the addition of sodium carbonate but not by the magnesium nitrate usually employed for the purpose. Leavitt and LeClerc (11) studying losses of phosphorus from wheat found ash higher at "low redness" than at "redness," 46 per cent higher values for phosphorus at "low redness" than at "redness," and 30 per cent lower values for phosphorus from samples ashed in the back of the muffle than from those in the front. Such losses were prevented with addition of calcium acetate, and if temperature was lower than "redness" loss of phosphorus was small even with omission of calcium acetate. Ash from wheat is acid and ash from plant material used in these experiments alkaline. No loss of phosphorus was found at 650° C. for the latter with ordinary ashing (Table III), and practically no change when ashed with sulphuric acid (Table VIII).

Additions of the so-called "aids to ashing" (Table VI) were found of little value when the ashing was made at 450° C. Addition of sulphuric acid to the sample before ashing gave a marked increase in the ash. This increase is no doubt due to the conversion of carbonates and chlorides to sulphates of higher molecular weight with a resultant loss in both carbonates and chlorides. Since both the carbonates and chlorides of potassium and calcium are stable at 450° C. while only those of magnesium are converted to the oxide there is little purpose in converting all of these salts to the respective sulphates by the addition of sulphuric acid. Use of these substances for low temperature ashing appears to be unnecessary and is not recommended.

Total base determinations as a means of securing an estimate of base content of plant tissue was not satisfactory. If phosphorus were removed the analysis would be more accurate. An assumption of complete conversion of bases is not correct since phosphorus is not volatilized (Table VIII), hence there is an error in computation. Total base values can not be substituted for ash in evaluating the mineral content.

METHOD PROPOSED FOR ASHING

Only tissue from plants protected from spattering of soil by rain or watering should be used. The dry material is finely ground and, if a metal mill is used for grinding, any particles of metal are removed with a small magnet. Small weighing bottles are loosely filled with the material, placed in an oven at 100° C. for two hours, removed and allowed to cool over calcium chloride. Portions of 0.3 to 0.5 g. are then weighed preferably by difference in weight of bottle before and after removal of material into tared porcelain capsules that have been cleaned well previously, burnt in a muffle at 650° C. and permitted to cool in a desiccator over calcium chloride. The material in the capsules is now flamed with a lighted match before being placed in the tube for ashing, since some tissues are of such composition as to ignite explosively upon heating. The furnace should be of the combustion tube type with the heating units completely encircling the tube and with resistance units adjusted to give an operating temperature of 450° C. The capsules are placed into a metal holder and so placed in the tube that each capsule is equidistant from the center of the tube. The tube is now inserted into the hot furnace, the center of the tube coinciding with the center of the furnace. A cork holding the lead from an oxygen tank is now placed in the tube and a rapid flow of dry, washed oxygen, passing through a bubble counter, is led over the samples in order to avoid explosions. After 15 minutes the rate of flow of oxygen is reduced to approximately 60 bubbles per minute. After eight hours the samples are removed, placed in a desiccator over calcium chloride, permitted to cool, and weighed.

SUMMARY

Ashing of tissues of tomato, buckwheat, and tobacco plants in a muffle at 650° C. for periods of 8 and 16 hours was compared with ashing in a combustion tube furnace for the same periods at 450° C. with oxygen. Differences in ash content, volatilization of constituents, and variation were observed.

Variation between duplicates and variation with repetition of ashing was less for ashing in the tube at 450° C. with oxygen than for the muffle at 650° C. Greater losses of ash were found with increasing temperatures but little correlation with increased periods of heating. Higher ash content was found at the lower temperature; the difference consisted of increased carbonate and chloride content and higher content of volatile inorganic constituents.

Amount of volatilization varied both with plants of the same species grown under different conditions, and with different species.

A survey was made of the components lost when the sulphates, car-

bonates, and chlorides of potassium, calcium, and magnesium were heated to 450, 650, 700, and 750° C. It was found that none of these constituents were lost when heated to 450° C. except chlorides of magnesium. At the higher temperatures both chlorides of potassium and calcium and the metals themselves are lost by sublimation. All sulphates were found stable at all temperatures.

Addition of chemicals, and especially sulphuric acid, to aid ashing resulted in higher values for the ash. In the case of the acid this increase was found due to the conversion of chlorides and carbonates to sulphates with a resultant increase in molecular weight.

Total base determinations were found to be unsatisfactory due to nonvolatilization of phosphorus and hence incomplete conversion of bases to sulphates.

A method for ashing, which was found to be superior to the usual method employed for the ashing of plant material, is described and proposed as a substitute for the old method.

LITERATURE CITED

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. COMMITTEE ON METHODS OF ANALYSIS. Collaborative work on ash. *In* Report. Cereal Chem. **3**: 267–276. 1926.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. COMMITTEE ON EDITING METHODS OF ANALYSIS. Official and tentative methods of analysis. 3rd ed. 593 pp. Washington, D. C. 1930.
3. BIBLE, C. M. Potash analyses. Indus. & Eng. Chem. Anal. Ed. **4**: 234–235. 1932.
4. BONNEAU, LUCIEN. Recherches sur la composition du cobaltinitrite de potassium-sodium. Bull. Soc. Chim. France, Sér. **45**: 798–808. 1929.
5. BRENDEL, G. L. Oxygen-acetate method of ash determination of flour. Cereal Chem. **3**: 222–226. 1926.
6. COLEMAN, D. A., and ALFRED CHRISTIE. Some observations on making ash determinations. Cereal Chem. **2**: 391–397. 1925.
7. HALD, PAULINE M. The determination of the bases of serum and whole blood. Jour. Biol. Chem. **103**: 471–494. 1933.
8. KIRNER, W. R. Quantitative organic microchemical analysis in industrial problems. Indus. & Eng. Chem. Anal. Ed. **5**: 363–369. 1933.
9. KLEIN, G. Handbuch der Pflanzenanalyse. Bd. 2, Spezielle Analyse 1 teil. 973 pp. Julius Springer, Wien. 1932.
10. KRAMER, BENJAMIN, and FREDERICK F. TISDALL. A clinical method for the quantitative determination of potassium in small amounts of serum. Jour. Biol. Chem. **46**: 339–349. 1921.
11. LEAVITT, SHERMAN, and J. A. LECLERC. Loss of phosphoric acid in ashing of cereals. Jour. Amer. Chem. Soc. **30**: 391–394. 1908.
12. LOCKHART, L. B. Ignition losses in potash analyses of triple superphosphate mixtures. Indus. & Eng. Chem. Anal. Ed. **3**: 407–408. 1931.
13. MCCLENDON, J. F., and ROE E. REMINGTON. The determination of traces of iodine. II. Iodine in vegetables. Jour. Amer. Chem. Soc. **51**: 394–399. 1929.
14. PIERRE, W. H. Determination of equivalent acidity and basicity of fertilizers. Indus. & Eng. Chem. Anal. Ed. **5**: 229–234. 1933.

15. POPOFF, STEPHEN, and E. W. NEUMAN. Microscopic examination of precipitates as an aid to precise analysis. Indus. & Eng. Chem. Anal. Ed. **2**: 45–54. 1930.
16. POTTS, R. B. Use of oxygen in the quick determination of ash. Cereal Chem. **8**: 232–233. 1931.
17. REMINGTON, ROE E., J. F. MCCLENDON, and HARRY VON KOLNITZ. The determination of traces of iodine. V. Further refinements of technique. Jour. Amer. Chem. Soc. **53**: 1245–1249. 1931.
18. REMINGTON, ROE E., J. F. MCCLENDON, HARRY VON KOLNITZ, and F. BARTOW CULP. The determination of traces of iodine. IV. Iodine in small quantities of thyroid and other tissues. Jour. Amer. Chem. Soc. **52**: 980–985. 1930.
19. VAN RYSSELBERGE, PIERRE J. Quantitative determination of potassium by sodium cobaltinitrite method. Indus. & Eng. Chem. Anal. Ed. **3**: 3–4. 1931.

EFFECT OF LIGHT AND DARK ON RESPONSES OF PLANTS TO GROWTH SUBSTANCES

P. W. Zimmerman and A. E. Hitchcock

Several earlier reports have dealt with the response of plants to growth substances while in light (4, 5, 6, 8, 9, 10). It was evident from the first that varying the environmental conditions changed the capacity of plants to respond when treated with any of several synthetic compounds commonly called growth substances or plant hormones. Variations in effects were noted with plants in different stages of maturity, similar plants at different seasons of the year, and plants in direct sunlight compared with those in shade. In order to modify the plant environment in a way that the various factors could be studied separately, plants were maintained in different light intensities and in dark chambers to prepare them for the experimental studies. The present paper reports the variations in response of several species of plants to growth substances while being kept in different light intensities and in dark for long periods of time. The principal responses of plants to growth substances with which this paper is concerned are local acceleration or retardations of growth resulting in negative (away from the place where the material was applied) or positive (toward the treated side) bending of stems or leaves, recovery of responded parts, the loss of capacity to make geotropic responses, local induction of roots, and the effects of environmental conditions with especial reference to light. Several earlier papers gave detailed description of responses in general (2, 4, 8, 9, 10).

MATERIALS AND METHODS

The plants used for experimental purposes were grown in the greenhouses in pots so they could be moved to the desired location. For most of the experiments the following plants were used: tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L. var. Turkish), Jerusalem artichoke (*Helianthus tuberosus* L.), Irish potato (*Solanum tuberosum* L.), African marigold (*Tagetes erecta* L.), sunflower (*Helianthus debilis* Nutt.), and dahlia (*Dahlia variabilis* Desf.). In addition to greenhouse plants, tubers of artichoke and potato, and storage roots of dahlia were planted in soil and etiolated plants grown entirely in the dark.

To determine the degree of response to application of the growth substances, the angles made by the upper side of the petiole and the adjacent stem were measured with a protractor immediately before and at intervals after treatment. Tomato plants were used for this purpose.

The following growth substances were used: alpha-naphthaleneacetic

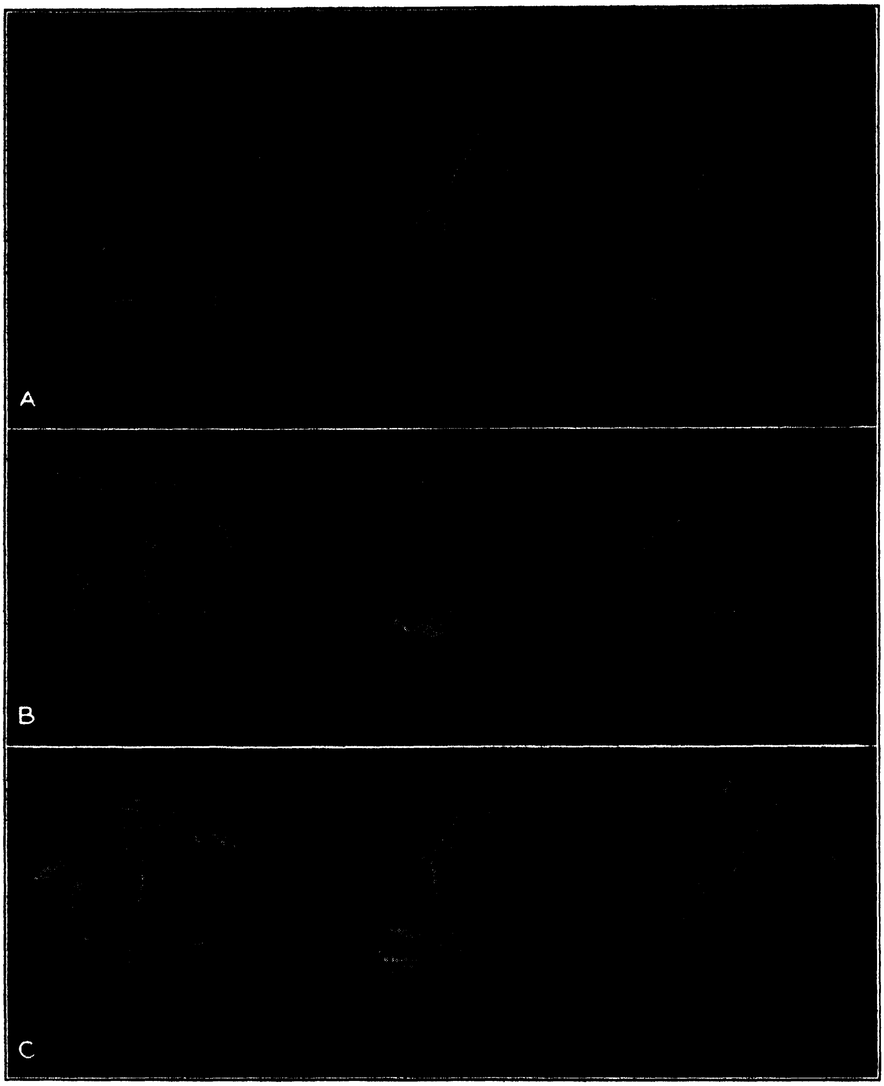


FIGURE 1. Tomato plants showing the effect of being in the dark five days in December, prior to treatment with indolebutyric acid. All plants were treated on the left side of the stem and on the upper side of adjacent petiole. A. Normal response of plants in greenhouse. Left: control. Middle: treated with 1% indolebutryic acid. Right: treated with 0.1% indolebutyric acid. B. Plants five days in the dark before treatment. Left: control. Middle: treated with 1% indolebutyric. Right: treated with 0.1% indolebutyric acid. Photographed after 24 hours. C. After being photographed, the plants shown in B were transferred to the greenhouse and then photographed again 24 hours later to show the change from positive to negative response induced by the aid of light.

acid, indoleacetic acid, indolebutyric acid, indolepropionic acid, and phenylacetic acid; and the methyl and ethyl esters of the foregoing acids.

The preparations used for treatments were made by mixing the growth substances with lanolin. The percentage concentrations most commonly used were 0.01, 0.05, 0.1, 0.5, and 1.0 in lanolin. To induce leaf epinasty the preparations were applied with a glass rod to the upper side of the petiole; stem bending was induced by applying the material along one side.

Microchemical methods were employed to make qualitative determinations for starch, sugar, and nitrates with the aid of a microscope. Flückiger's reagent (copper tartrate with KOH) was used for sugar and iodine solution for starch. Diphenylamine was used for the nitrate tests.

EXPERIMENTAL RESULTS

Lanolin preparations containing low concentrations of growth substances induce negative bending of stems or leaves. This response is due to local acceleration of growth where the material is applied. High concentrations induce positive bending due to local retardation of growth. The exact concentrations causing these responses vary with the growth substances concerned. For example, 0.002 per cent to 1.0 per cent of α-naphthaleneacetic acid in lanolin caused negative bending of tomato stems and leaves while 2.0 per cent or more caused positive bending. Somewhere between 1.0 per cent and 2.0 per cent there should be found a concentration which would not change the rate of growth and therefore cause no bending.

Typical negative bending of plants in light due to local application of indolebutyric acid is illustrated in Figure 1 A. The bending responses occur within an hour, reaching the maximum degree within six to ten hours. When treated with low concentrations in light, the affected parts make considerable recovery (return toward their original position) within 24 hours. The degree of recovery varies with the concentration, being nearly complete with the lowest concentrations. Very little recovery occurs when the plants are treated with 1.0 per cent lanolin preparations of the most effective substances. In this case the affected parts swell and produce roots in five to ten days if the plants remain in light. If, however, the plants are moved at the time of treatment to heavy shade or continuous darkness, the degree of recovery and rooting responses are modified.

MODIFICATIONS IN RESPONSE TO ENVIRONMENT

Plants of one variety growing under the same conditions made uniform responses when treated alike with a given concentration of growth substance. Leaves treated with 0.01 per cent in lanolin grew downward for a few hours and then returned toward the original position. Measurements showing the degree of response and tendency of the leaves to recover when

in light are shown in Table I. The data show that when low concentrations of the substances are applied to the upper side of petioles of plants in the greenhouse, the leaves grow downward for six to ten hours but tend to recover in 24 hours.

TABLE I

EPINASTY INDUCED WITH LANOLIN PREPARATIONS OF INDOLEACETIC ACID WHILE PLANTS WERE IN A GREENHOUSE*

Treatment	Measurements of angles stated in degrees					Per cent change in 6 hrs.	Per cent change in 24 hrs.
	Original angle	After 6 hrs.	After 24 hrs.	Change in 6 hrs.	Change in 24 hrs.		
	57	117	83	60	26	105	46
	46	107	71	61	25	132	54
	41	109	97	68	56	166	137
	48	121	82	73	34	152	71
Indoleacetic acid 0.01% in lanolin	48	102	94	54	46	112	96
	49	92	64	43	15	88	35
	45	121	61	76	16	169	36
	54	78	75	24	21	44	39
	48	90	86	42	38	88	79
	42	92	56	50	14	119	33
Mean	47.8	102.9	76.9	55.1	29.1	117	63
	52	104	78	52	26	100	50
	65	103	57	38	−8	59	58
	53	126	100	73	47	138	89
	48	80	56	32	8	67	17
Indoleacetic acid 0.005% in lanolin	56	81	87	25	31	45	55
	45	81	66	36	21	80	47
	53	82	69	29	16	55	30
	54	85	78	31	24	57	44
	47	88	68	41	21	87	45
	40	82	56	42	16	105	40
Mean	51.3	91.2	71.5	39.9	20.2	79	47
	50	51	45	1	−5	2	−10
	49	50	48	1	−1	2	−2
	43	44	43	1	0	2	0
	50	51	45	1	−5	2	−10
	49	50	48	1	−1	2	−2
Control	50	52	57	2	7	4	8
	54	58	53	4	−1	7	13
	61	60	60	−1	−1	16	26
	48	51	45	3	−3	6	−6
	43	45	45	2	2	5	12
Mean	49.7	51.2	48.9	1.5	0.8	4.8	8.9

* Two leaves per plant were selected for the treatments. Measurements (in degrees) of angles between the stem and petiole were taken with protractor at time of treatment and again after 6 and 24 hours. Induced growth caused downward movement of leaves. Recovery was indicated by return of leaves toward their original position.

There was considerable difference in the degree of bending induced from treatment on clear and cloudy days. Responses were modified by

moving the plants from the greenhouse to a dark room when the growth substance was applied. Darkness had the effect of increasing the degree of response and preventing recovery. Table II shows that plants in the dark had more pronounced bending in 24 hours than in seven hours in

TABLE II

EFFECT OF KEEPING TOMATO PLANTS IN DARK 18 HOURS BEFORE TREATMENT AND 24 HOURS DURING RESPONSE*

Location of plants	Hours after treatment	Per cent increase in angles after treatments. Average of two petioles				
		Check	Per cent indoleacetic acid in lanolin			
			0.0006	0.0012	0.0025	0.005
Greenhouse	7	8	35	39	80	70
	24	15	10	24	60	45
Dark room	7	14	32	123	105	153
	24	2	34	135	175	264

* The weather was clear 24 hours prior to and during the experiment. The plants were moved to the dark room at 4:30 P.M. and treated the next morning at 9:30 A.M.

contrast to similar plants in the greenhouse on a clear day. This held also for plants which had been in a dark room four days prior to the time the substance was applied. Similar plants returned to the light after four days in the dark tended to recover after treatment as did plants kept continuously in the greenhouse. Figures showing the results of the experiment are given in Table III.

TABLE III

EFFECT OF 4 DAYS OF DARK PRIOR TO TREATMENT TO INDUCE EPINASTY OF LEAVES, AS COMPARED TO PLANTS IN LIGHT AND PLANTS TRANSFERRED TO LIGHT AFTER 4 DAYS IN DARK

Location of plants	Treatment (indoleacetic acid given as per cent in lanolin)	Per cent change in angle after treatment. Figures represent average of 4 leaves	
		After 5 hrs.	After 24 hrs.
Greenhouse continuously	0.005	52	19
	0.01	71	35
	Control	8	18
Dark room 4 days	0.005	89	91
	0.01	89	120
	Control	2	8
Dark room 4 days then in greenhouse after treatment	0.005	95	44
	0.01	54	35
	Control	35	27

Leaves of young actively growing plants made a more pronounced bending response to a given concentration of the substances than similar plants that were slow growing due to shortage of fertilizer. Shaded plants showed more epinasty (downward bending) of leaves five hours after treatment than similar plants in direct light.

EFFECT OF PROLONGED DARK PERIODS PRIOR TO TREATMENTS

Tomato Plants

Plants in light had a tendency to recover from induced epinasty of the leaves or bending of the stems while those in the dark did not. The effect of the first 24 hours of continuous darkness on tomato plants 6 to 8 inches in height was to increase the degree of response to a given concentration of growth substance as compared with that of a similar plant in light (Tables II and III). Plants left in the dark continuously for four days before treatment with 0.05 and 0.1 per cent indoleacetic acid showed less bending than those in the dark for one and two days. The increasing loss of capacity of tomato plants to respond to applications of the substances continued for eight to ten days of darkness in summer, finally reaching a condition where no bending could be induced.

The sensitivity of plants to a given concentration of the substance varied after the first 24 hours in the dark. One per cent preparations of naphthaleneacetic or indoleacetic acid when applied locally to plants in light accelerated growth, causing negative bending. The same preparations applied to tomato plants kept in the dark for 48 hours or more induced positive bending, due to local retardation of growth. Lower concentrations (0.1 per cent or less) of the substances, however, caused negative bending of similar plants remaining in the dark for eight days during September or five days in December.

The influence of light was evidenced by the fact that plants in dark giving a positive response to a local application of the substances changed to negative shortly after they were placed in the light (Fig. 1B and C, Table III). After a prolonged stay in the dark, plants finally failed to respond when treated with the most favorable concentrations of growth substances. These plants responded, however, when returned to light if they had not been badly damaged by the long stay in the dark.

Tobacco Plants

Actively growing tobacco plants six inches in height responded similarly to tomato plants when placed in the dark. Older plants, however, tolerated continuous darkness for longer periods, elongating for six weeks or more, depending on the original size. Plants 18 inches in height, placed in a dark room in November, continued stem elongation for 30 days. While

they were in a growing condition, local acceleration of growth could be obtained by application of the growth substances. Reducing sugars and starch were found after the large plants had been in the dark for 40 days. This is in contrast with tomato plants in the dark. Tobacco plants also produced adventitious roots when treated in the dark, whereas tomato plants did not. Light was not necessary for induction of roots on stems of large tobacco plants in the dark.

Plants with Storage Organs

Dahlias, potatoes, and Jerusalem artichokes were tested for their capacity to respond to growth substances after long periods in complete darkness. The plants were grown from roots or tubers in the dark room or started in the greenhouse and then the green plants transferred to a dark room.

Dahlia plants started in light responded to the growth substances (0.1, 0.5, and 1.0 per cent naphthaleneacetic acid and 0.5 and 1.0 per cent indoleacetic acid) when treated near the growing regions after 35 days in the dark room. In addition to the bending response, adventitious roots were induced where the stems were treated with 1.0 per cent lanolin preparations of indoleacetic acid or 0.1 to 0.5 per cent naphthaleneacetic acid.

At the end of 35 days, qualitative tests indicated the presence of reducing sugars.

Potatoes grown in soil in the dark from the first were tested at intervals over 60 days and found to respond (negative bending) when treated with a range of concentrations of naphthaleneacetic, indolepropionic, and indoleacetic acid from 0.01 to 1.0 per cent in lanolin. The etiolated vines were repeatedly removed and though the new ones were greatly reduced in diameter, they responded where treated. As the new shoots became spindly, they were more sensitive and made positive bends to high concentrations of the substance but the low concentrations (0.01 per cent) induced negative bending.

Potato stems from tubers grown for 25 days in the dark were etiolated and practically leafless. In this condition there was present an abundance of sugar and starch as indicated by microchemical methods. The stems exhibited negative geotropism when placed in a horizontal position. While in a horizontal position they were made to grow downward (resembling positive geotropism) by treatment on the upper side with 0.1 to 1.0 per cent lanolin preparations of the growth substances. Natural negative geotropism and induced "positive geotropism" are shown in Figure 2 A.

Swelling and retardation of stem elongation of potatoes similar to that reported for sweet pea stems and that induced by unsaturated hydrocarbon gases (2, 10) were produced by a wide range of concentrations of

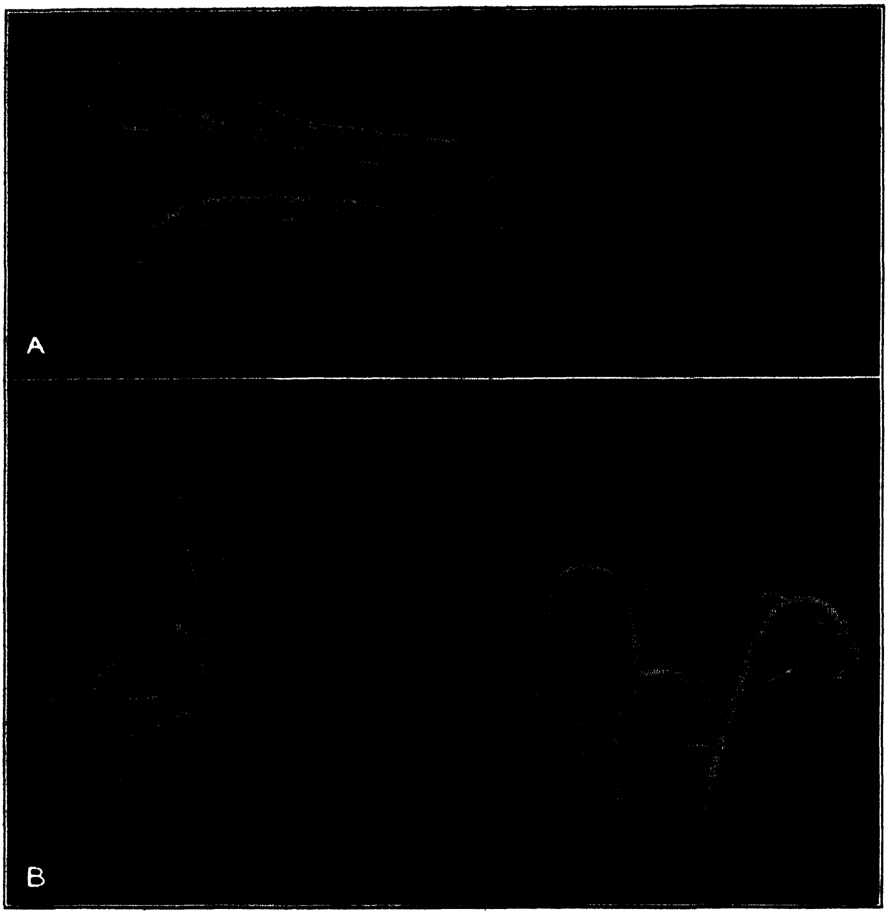


FIGURE 2. Response of potato plants to growth substances while growing in the dark. A. Etiolated potato plants grown for 25 days in the dark, then placed in a horizontal position, at which time the lower six shoots were treated on the upper side with growth substances. The others were left as controls. Photograph was taken 48 hours after the plants were placed in a horizontal position. B. Plants started in light, then transferred to the dark room and left for five days prior to treatment with growth substances. Left: Tall straight plants not treated. Four plants on the extreme left treated as follows: 1% indoleacetic, 0.1%, 0.5%, and 1% naphthaleneacetic acid. Right: Two plants pointing to left treated with 0.5% and 1% indoleacetic acid. Two plants pointing to right treated with 0.5% and 1% naphthaleneacetic acid. Photograph taken 48 hours after treatment.

growth substances (Fig. 2 B). The ultimate results are retardation in one direction (elongation) with an associated increase in the other direction (diameter).

Roots were induced where potato stems were treated with lanolin preparations containing 1.0 per cent indoleacetic acid or 0.1 per cent naphthaleneacetic acid. Best rooting occurred when the first set of shoots were treated. As the vines became spindly they lost their capacity to produce adventitious roots.

Both starch and sugar were detected in potato tissue of plants in the dark as long as growth continued, which in some cases was 81 days. During this time bending was induced by growth substances.

Potato plants grown in the greenhouse from different sized seed pieces varied in endurance when transferred to the dark room according to the size of the mother tubers and the length of time grown in the greenhouse. Those with large tubers lasted longest, but not as long as plants from similar tubers which were started and kept continuously in the dark. Plants from seed pieces sized approximately 2 cc. responded to treatment for only five to eight days in December after being transferred to the dark room. Their capacity to respond to the growth substances resembled that of small tomato plants.

Leaves of potato plants transferred from light to dark had a tendency to become chlorotic more readily than tomato or tobacco leaves. This was especially striking with large plants placed in the dark.

Artichoke plants from large tubers were started in the dark and grown continuously for 60 days without loss of capacity to respond to growth substances, as indicated by negative bending and production of adventitious roots where the stems were treated. The white, etiolated stems grew from three to four feet long during the first month in the dark. As these were removed new branches grew but they were not as thrifty as the first crop. Though somewhat spindly, at the end of 51 days the stems still responded to applications of the growth substances. The 1.0 per cent concentration of indoleacetic acid caused positive bending of the spindly sprouts but 0.01 to 0.1 per cent concentrations accelerated growth, inducing negative bending.

As with potatoes and dahlias, roots were induced on artichoke stems where treated with 1.0 per cent indoleacetic and 0.1 to 0.5 per cent naphthaleneacetic acid. Best rooting occurred on the first crop of thrifty shoots. Roots could be induced also on spindly shoots though they were not as abundant as on the early thrifty shoots.

Reducing sugars were detected by microchemical methods throughout the entire time (60 days) the artichokes were grown in the dark. Nitrates also were present in etiolated stems and leaves though not as abundant as with plants transferred from light to the dark for a few days.

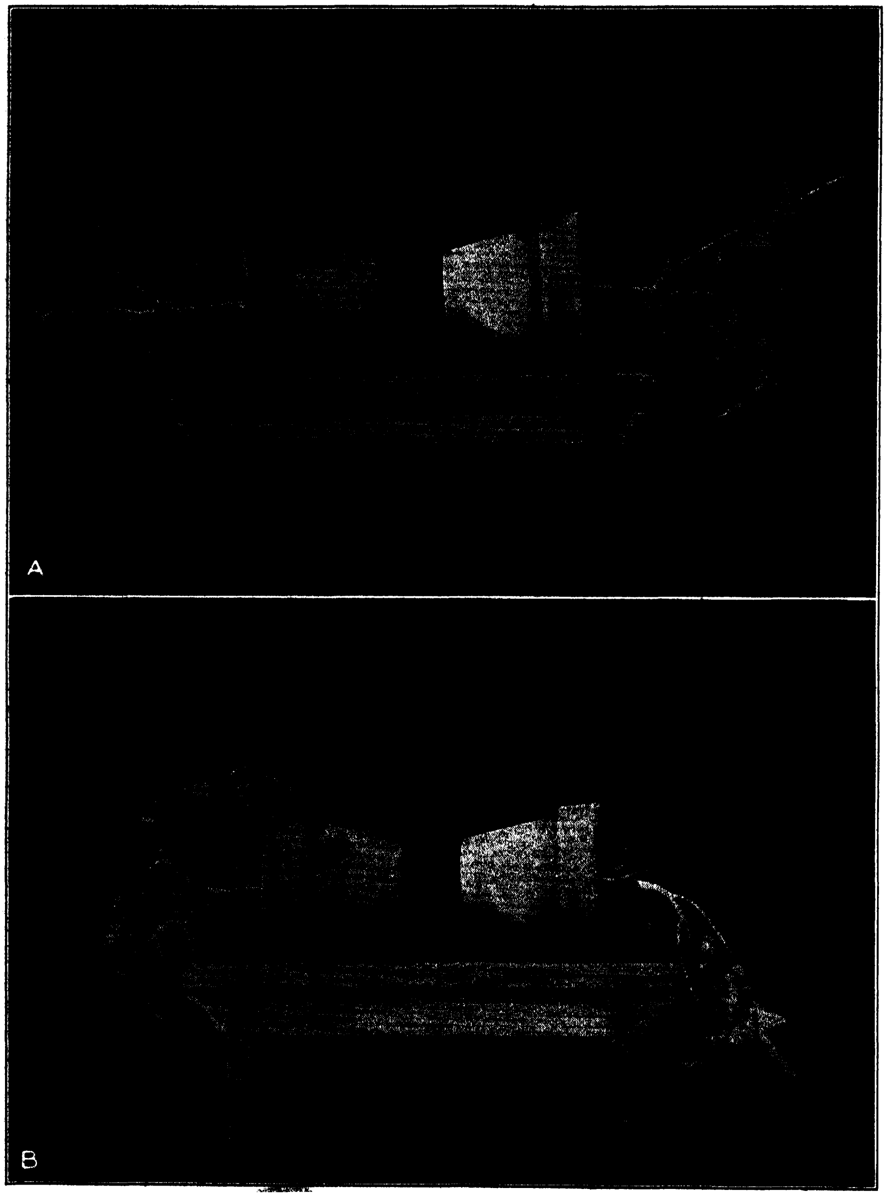


FIGURE 3. Tomato plants placed in the dark December 12th. A. After four days in the dark, the plants were put in a horizontal position and photographed 24 hours later to show that they had lost their capacity to respond to the force of gravitation. B. Same plants as in A, 24 hours after treatment with lanolin preparations containing 0.1% indolepropionic acid. Left: treated on under side. Right: treated on upper side. Photograph taken 18 hours after treatment.

Leaves of etiolated shoots, though much smaller than those of plants in light, responded to the same concentration of growth substances as the stems.

Artichoke plants growing in light tolerated higher concentrations of substances than etiolated shoots in the dark. If, however, plants grown in light until the shoots were two to three feet in height, were transferred to dark, they became etiolated and as sensitive as those grown in dark. As with potatoes, however, artichoke plants grown to a height of two feet and then transferred to the dark did not last as long as those grown in the dark from the first.

Loss of Capacity to Respond to Gravity

When normal plants were placed in a horizontal position they were able, through some natural mechanism, to right themselves; that is, at least a part of the stem grew upward (negative geotropism). After five days in a dark room during December, six-inch tomato plants lost their power to right themselves while lying for 24 hours in a horizontal position. At this time, however, they still had the capacity to respond to 0.1 per cent indoleacetic acid. While in a horizontal position if treated on the upper side with growth substances, these plants were made to grow downward (resembling positive geotropism); if treated on the under side they grew upward (resembling negative geotropism). Figure 3 illustrates the failure to respond to gravity while they were still able to respond to the growth substances.

The age and condition of the plants at the time they were placed in darkness affected the length of time they could be kept without light and still respond to gravity or to the growth substances. During December, six-inch tomato plants failed to respond to gravity within four to five days and to the substances after six to seven days. Plants ten inches in height responded to gravity after seven days in the dark and to the substances after eight to ten days. Several varieties of plants transferred to a dark room lost their capacity to respond to the force of gravity while they could respond to growth substances. Marigold plants transferred from the greenhouse to the dark room on November 17 failed to exhibit negative geotropism on November 22. After 24 hours in a horizontal position they were, however, induced to bend by means of 0.01 per cent naphthaleneacetic or indoleacetic acid.

Sunflower plants in dark from November 22 to November 26 lost their power to respond to gravity but not to the growth substances.

Six-inch tomato plants in the dark from November 22 to November 27 responded to indolebutyric, indolepropionic, indoleacetic, and naphthaleneacetic acid but not to gravity. The experiment repeated with plants in the dark from April 22 to April 28 gave similar results. At this time the

methyl esters of the various acids were tested and found to be as effective as other growth substances.

As with tomato plants, small tobacco plants six inches in height lost their capacity to grow away from gravity if left in the dark during December for eight to ten days. After reaching this condition they still responded to the growth substances if tested before they were too badly damaged by the long stay in the dark. After losing their capacity to respond while in the dark they recovered sufficiently in light in 24 hours to bend where the preparations (0.1 per cent) had been applied.

Potato plants from good sized mother tuber pieces were started in light and then were transferred to the dark on November 25 when they were 10 to 12 inches in height. On December 9 some of the plants failed to right themselves while lying in a horizontal position for four days. While in this condition they still responded to the indole and naphthalene growth substances. On the other hand, potato plants grown in the dark from similar size pieces of tubers kept their capacity to respond to gravity for 60 days.

Permanent Wilting in the Dark

Small tomato plants kept continuously in a dark room for five to seven days in December or seven to ten days in summer showed signs of permanent wilting. The first symptoms did not show uniformly over the plant nor did all plants start wilting at the same time. The third or fourth leaf from the tip usually wilted before the old or young leaves. Once in this condition they could not be revived by light, moisture, or other factors most favorable for growth. At the present time there seems to be no explanation for this peculiar phenomenon. The accumulation of nitrates might be involved, since qualitative tests indicated increasingly large amounts of nitrates in leaf tissue from the time the plants were started in the dark. Conversely, carbohydrates decreased from the start and when permanent wilting occurred no starch or sugar could be detected in the tissues. This condition indicates that nitrate accumulation and carbohydrate loss are at least associated with permanent wilting.

DISCUSSION

The data presented under "Experimental Results" show that light in some way modifies the capacity of plants to respond to growth substances. Just what happens in the living cells to bring about the modification is not clear. Plants left in light show their maximum response to treatment in six to ten hours and then the affected parts tend to return toward their original position. They make almost complete recovery when treated with low concentrations (Table II) but little to none when a 1.0 per cent concentration of the substances is used. In the dark the degree of response of treated parts increases up to 24 hours with no recovery thereafter. If the

magnitude of response is due to the combined effect of the growth substance and the natural hormone, which according to Avery (1) rapidly disappears in the dark, then the relative difference in the total material on the two sides of a treated stem or leaf should increase as soon as the plants are moved from the light to the dark. That is, the lanolin preparation applied to one side of the organ remains as a source of supply whereas the natural hormone disappears.

Sensitivity of the plant tissues to growth substances increases with continued stay in the dark. For example, 1.0 per cent indolebutyric acid induces positive bending of tomato stems and leaves of plants left in the dark five days, whereas plants in light tolerate two to three per cent. If, however, after dark plants show positive bending they are moved to light, the treated parts exhibit negative bending without further application of the growth substance (Fig. 1). If plants from the dark are treated and then transferred to light they at first make a more pronounced response than normal plants in the light, but later tend to recover in contrast with plants left in the dark. The recovery may be aided by the production of natural hormone when the plants are in the light (1).

Darkness, though it at first increased the capacity of plants to respond, if prolonged, caused a decrease and finally complete loss of the power to respond. Similarly the plants failed after a few days in the dark to respond to the force of gravitation. At this time, however, they still responded to treatment with the various growth substances (Fig. 3).

If the theory (7), that the upward growth of plants placed in a horizontal position is due to the redistribution of natural hormones, is correct, plants losing their capacity to grow upward have presumably lost all their natural hormones. Plants in this condition should make good test objects to determine which chemical compounds might properly be placed in the category of "growth substances." On that basis the methyl and ethyl esters of naphthaleneacetic acid, indolebutyric acid, indolepropionic acid, indoleacetic acid, and phenylacetic acid, as well as the acids themselves, must be considered as true growth substances, since they were all effective on plants which had lost their capacity to respond to the force of gravitation. Therefore the criticism that some of the compounds previously reported as growth substances only interfere with movement of natural hormones (3) can hardly apply.

Avery (1) found that tobacco plants large enough to have 10 to 18 leaves lost all of their natural auxin after five days in the dark. In this connection it is interesting to point out the fact that tobacco plants 18 inches in height placed in the dark in November 1935 showed stem elongation for more than 30 days during which time they also responded to treatment with growth substances. If there can be no growth without the presence of hormones and if tobacco plants lose their natural hormones after five days

in the dark, it is difficult to account for the growth of the plants in question. In this connection it is interesting, also, to consider plants with special storage organs. Potatoes, for example, grew for more than 80 days in the dark during which time they retained the capacity to respond to the force of gravitation and to the growth substances.

Potatoes, artichokes, and dahlia plants grown in the dark from time of planting differed from those that were started in light and later transferred to the dark. Those grown to a height of 12 to 18 inches in the greenhouse were unable to tolerate darkness as well as plants grown from the first in a dark room. The transferred potatoes and artichokes lost their capacity after 15 to 20 days to respond to either gravity or the growth substances, whereas the dark plants responded after 60 to 80 days. It appears that the plants growing in light tax the mother tubers to a greater extent than those in the dark.

Adventitious roots could be induced on plants of tobacco, potato, artichoke, and dahlia while in the dark but the growth of these was much less than that of plants in the light.

SUMMARY

Several species of plants were studied under a variety of conditions to determine the effect of environment on their capacity to respond to treatment with the following growth substances: α-naphthaleneacetic acid, indoleacetic acid, indolebutyric acid, indolepropionic acid, phenylacetic acid, and the methyl and ethyl esters of these acids.

The degree of response due to acceleration of growth where treated with the substances varied with the conditions under which the plants were grown, those in bright light tolerating higher concentrations and making less response than similar plants in deep shade.

Plants in light treated with low concentrations of the substances made their maximum response in six to ten hours and then the leaves and stems tended to recover, that is, return toward their original position. Plants transferred to the dark at the time of treatment showed a more pronounced response than those in the light, and the treated parts did not tend to recover.

Prolonged dark periods caused the plants to become more sensitive to the growth substances. Lanolin preparations containing 1.0 per cent of the growth substances, causing pronounced negative response in the light, induced positive response on plants in the dark. These latter plants, however, exhibited negative bending shortly after they were transferred back to light.

Stems of tomato plants five inches in height lost their capacity to respond to the force of gravitation (negative geotropism) after five days in the dark during December. Larger plants lasted for a longer period of time.

After the plants lost their capacity to grow away from the center of the earth, they still had the power to respond to growth substances. After a few more days in the dark, however, the plants lost their capacity to respond to growth substances.

Tobacco plants 18 inches in height retained their capacity to respond to both gravitation and to growth substances for a period of 30 days in darkness.

Potato, artichoke, and dahlia plants grown in the dark from the first produced etiolated stems which retained their capacity to respond to the force of gravitation and growth substances for 60 to 80 days after planting. Plants of the same varieties grown first in light to a height of 12 to 18 inches and then transferred to the dark room did not retain their capacity to respond as well as plants grown continuously in darkness.

Adventitious roots were induced on tobacco, artichoke, and potato plants while in darkness, though the newly formed organs did not continue to grow as well as those induced in light.

Permanent wilting of leaves, decrease of carbohydrates, and increase of nitrates in the tissues were associated with the loss of capacity of tomato plants to respond to the force of gravitation and to growth substances while in the dark.

LITERATURE CITED

1. AVERY, GEORGE S., JR. Differential distribution of a phytohormone in the developing leaf of *Nicotiana*, and its relation to polarized growth. Bull. Torrey Bot. Club **62**: 313–330. 1935.
2. CROCKER, WILLIAM, A. E. HITCHCOCK, and P. W. ZIMMERMAN. Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. **7**: 231–248. 1935.
3. HAAGEN SMIT, A. J., and F. W. WENT. A physiological analysis of the growth substance. Proc. Akad. Wetensch. Amsterdam, Sec. Sci. **38**: 852–857. 1935.
4. HITCHCOCK, A. E. Indole-3-*n*-propionic acid as a growth hormone and the quantitative measurement of plant response. Contrib. Boyce Thompson Inst. **7**: 87–95. 1935.
5. ——— Tobacco as a test plant for comparing the effectiveness of preparations containing growth substances. Contrib. Boyce Thompson Inst. **7**: 349–364. 1935.
6. HITCHCOCK, A. E., and P. W. ZIMMERMAN. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. **7**: 447–476. 1935.
7. KEEBLE, FREDERICK, M. G. NELSON, and R. SNOW. The integration of plant behaviour. IV. Geotropism and growth-substance. Proc. Roy. Soc. [Lond.] B. **108**: 537–545. 1931.
8. ZIMMERMAN, P. W., and A. E. HITCHCOCK. The effect of subjecting plants to light and dark prior to treatment with growth substances. Amer. Jour. Bot. **22**: 910. 1935.
9. ZIMMERMAN, P. W., A. E. HITCHCOCK, and FRANK WILCOXON. Several esters as plant hormones. Contrib. Boyce Thompson Inst. **8**: 105–112. 1936.
10. ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

PROPAGATION OF LYCOPODIUM. III. SPORE GERMINATION

Florence L. Barrows

Experiments on the germination of *Lycopodium* spores were described in a previous paper (1). Later data are now available on some of these same cultures which were continued to March 1936.

LYCOPODIUM COMPLANATUM VAR. FLABELLIFORME FERNALD

The previous report (1), Table III, p. 272, gave the percentage of germination of *Lycopodium complanatum* var. *flabelliforme* Fernald spores which had been stored for five months at different temperatures and then cultured at room temperature on moist peat moss in petri plates for 12 months and 14 months. Another count was made on May 16 to 17, 1935 (1, p. 273) in which the percentage of germination did not show very marked changes from the count made previously in April. Through an error in calculations the per cent column as given in the previous report (1), Table III, was incorrect. The corrected figures are given in Table I, columns 2 and 3.

TABLE I

L. complanatum Spores Stored for Five Months at Different Temperatures; Per Cent Germination on Moist Peat Moss Feb. 1934 to March 1936

Storage temperatures	Feb. 1935*	Apr. 1935*	Mar. 1936
1. Room, light	7.7	9.8	19.3
2. Room, dark	11.1	12.1	19.0
3. Oven, 1° C.	18.4	16.3	18.1
4. Oven, 5° C.	9.7	12.4	15.3
5. Oven, 10° C.	11.2	11.6	13.6
6. Oven, 15° C.	7.2	12.4	16.8
7. Oven, 20° C.	10.4	14.0	16.0
8. Oven, 25° C.	8.1	6.8	15.6
9. Oven, 30° C.	1.1	7.2	8.8
10. Oven, 35° C.	8.9	7.7	14.7
11. Alternating 5° to 25° C.	14.0	15.5	17.2
Average	9.8	11.4	15.8

* Through an error in calculation of per cent germination, incorrect figures were published in Table III, 1935 (1), columns 4 and 5. Corrected figures are given above in columns 2 and 3.

Counts made March 16 to 17, 1936, when these same cultures were nearly 25 months old, showed more germination than any previous count. Percentage germination ranged from 8.8 to 19.3 with an average of 15.8 per cent. Figures for the different cultures are given in Table I. Spores were removed to a slide by means of a sterile platinum needle for each count and were not returned to the culture dishes.

Development of gametophytes. The amount of development of the gametophytes from germinating spores was about eight to ten cells at the maximum, and about the same stages as those found in the same cultures a year earlier, and previously illustrated (1, Fig. 1 A and B and Fig. 2 A). On May 16 to 17, 1935, cultures 1, 3, 5, 7, and 9 were inoculated with a fungus isolated from a gametophyte of *Lycopodium obscurum* and previously described (2). These inoculated cultures showed no marked differences from those not inoculated. Most of the gametophytes contained plump cells with oil globules along the cell walls as shown in Part I (1, Fig. 1 B). Occasionally cells were collapsed, but most of the gametophytes looked normal. Attempts to cultivate them after transfer to potato dextrose agar, 2 per cent maltose, filtrate of the fungus mentioned above added to agar, or in Beyerinck solution, were unsuccessful. The fungus developed very rapidly and soon overgrew the cultures so that it was almost impossible to recover any of the *Lycopodium* spores.

LYCOPODIUM OBSCURUM L.

The cultures of *Lycopodium obscurum* L. (1, p. 275–276) which had been stored at different humidities for two weeks, from November 6 to 20, 1933, were cultured on damp sphagnum from November 20, 1933 to March 17, 1936, about 28 months. In the previous report (1, p. 277) the number of germinating spores was small and no counts were made. After 28 months the number of germinations had increased markedly and counts were made of ten microscope fields for each spore culture as with *L. complanatum*. The range was from 12.5 to 27.3 and averaged 20.7 per cent for the ten cultures. Table II gives the actual counts and percentage germination of *L. obscurum*.

TABLE II

L. OBSCURUM SPORES STORED TWO WEEKS AT DIFFERENT HUMIDITIES; GERMINATION ON DAMP SPHAGNUM NOVEMBER 1933 TO MARCH 1936

Humidity	No. germ*	Total count*	Per cent germ.
1. Air dry	74	591	12.5
2. Over $CaCl_2$	25	199	12.5
3. 10 per cent	96	613	15.6
4. 25 per cent	175	739	23.6
5. 35 per cent	182	795	22.8
6. 50 per cent	5	27	18.5
7. 60 per cent	130	476	27.3
8. 75 per cent	96	459	20.9
9. 90 per cent	107	492	21.7
10. Over conc. H_2SO_4	113	434	26.0
Totals	1003	4825	201.4
		Average	20.1

* Counts of 10 fields of the microscope.

On May 17, 1935, cultures 1, 3, 5, 6, 8, and 10 had been inoculated with the endophytic fungus isolated from a *Lycopodium obscurum* gametophyte —the same fungus being used in the *L. complanatum* cultures. Very few fungus hyphae were present when the counts were made on March 17, 1936.

Development of gametophytes. Some cell divisions had occurred inside the spore coats, but many gametophytes had burst the spore coats and were protruding as previously shown (1, Figs. 1 C and 2 B and C). All the *L. obscurum* gametophytes examined looked normal. Rows of small oil globules were frequent along the newly formed cell walls. In several cultures germination had occurred inside sporangia still attached to the cones or strobili, the whole tissue of which had become much softened. Attempts to culture the germinating spores in 2 per cent maltose resulted in the recovery of the endophytic fungus from several of the inoculated cultures, but not in further development of the gametophytes.

ALCOHOL TREATMENT

After washing in 95 per cent alcohol for five minutes on October 24, 1935, freshly-harvested spores of *L. obscurum* were placed on moist peat moss, in sterile distilled water, and on potato dextrose agar at room temperature. By November 1, or within eight days, germination of 2-cell and a few of 3- or 4-cell stages were found in all three cultures.

Fresh *L. clavatum* L. spores treated the same way on October 24 had, by November 2, also developed a few 2- and 3-cell stages on peat moss, distilled water, 2 per cent maltose, and potato dextrose agar.

Fresh *L. complanatum* spores given the same kind of alcohol treatment on October 28 showed a few 2-cell stages on peat by November 4, one week later, but no germination in distilled water, 2 per cent maltose or on potato dextrose agar.

One week was the shortest period in which germination was obtained in any of these tests of *Lycopodium* spores. However, only small numbers were germinated in any of these cultures and none developed beyond the 4-cell stage, even after five months. On all the media except peat moss, fungi soon overgrew the cultures.

AGED SPORES

L. complanatum spores which had been stored two years in a cool room usually just above freezing, and then treated with 95 per cent alcohol, were sown in moist peat moss October 28, 1935. Up to March 27, 1936 no germination had appeared in either the treated lots or the controls.

L. complanatum and *L. lucidulum* Michx. spores stored for two years in a freezing room where the temperature was 0° C. or below, showed no germination after five months on moist peat moss from October 28, 1935 to March 27, 1936.

SUMMARY

1. Spores of *Lycopodium complanatum* var. *flabelliforme* Fernald which had been stored for five months at different temperatures and then cultured on moist peat moss for about 25 months at room temperature showed germination. The percentage of germination ranged from 8.8 to 19.3 with an average of 15.8 per cent.

2. *Lycopodium obscurum* L. spores on damp sphagnum for 28 months showed a range of 12.5 to 27.3 and an average of 20.0 per cent of germination. This slightly superior germination may account for the fact that larger numbers of gametophytes of this species have been reported than of most others (1, Table II, p. 271).

3. Development of the gametophytes in both species reached a 10-cell stage. The problem of securing more advanced development on artificial media remains unsolved.

4. Spores of *L. obscurum*, *L. clavatum* L., and *L. complanatum* treated for five minutes with 95 per cent alcohol gave a few germinations on several media within a week; but gametophytes developed only to the 4-cell stage.

5. Aged spores of *L. complanatum* and *L. lucidulum* Michx., which had been stored at just above 0° C. or constantly below 0° C. for two years failed to germinate after five months on moist peat moss.

LITERATURE CITED

1. BARROWS, FLORENCE L. Propagation of *Lycopodium*. I. Spores, cuttings, and bulbils. Contrib. Boyce Thompson Inst. **7**: 267–294. 1935.
2. ——— Propagation of *Lycopodium*. II. Endophytic fungus in gametophyte and sporophyte. Contrib. Boyce Thompson Inst. **7**: 295–309. 1935.

MOISTURE AND TEMPERATURE REQUIREMENTS FOR YAROVIZATION OF WINTER WHEAT

MARY LOJKIN

INTRODUCTION

Both temperature and light are important factors influencing the transition of plants from the vegetative to the reproductive stage. Winter cereals do not head during the first year if they are planted late in the spring and are thus deprived of the usual exposure to low temperature which the young plants receive when sown in the fall. The heading of winter cereals depends upon the date of sowing, the climate, and the variety used. For each variety of winter cereal there is a definite or critical date of planting which depends upon climatic factors. If sown before this critical date the plants of any winter variety will head during the first summer. If sown after this critical date the plants continue prostrate vegetative growth during the summer and head during the next year. The purpose of this work was to study the effect of low temperature treatments applied to the seed during the first stages of germination upon the length of the vegetative phase of the plant. Experiments were undertaken to determine the conditions necessary for the completion of the cold treatment of winter wheat such as the moisture content of seeds, the temperature, and the required time period of treatment, taking into consideration also the light and temperature conditions of the subsequent growth of the plant.

REVIEW OF LITERATURE

As early as 1857 Klippart (9, p. 757) observed that the processes bringing the plant to the reproductive stage under the stimulating effect of low temperature could take place not only in the well developed plant, but also in the seed in its first stages of germination. He reported that winter wheat could be converted into spring wheat by allowing the seed to germinate slightly and then preventing it from vegetating by keeping it frozen until the time of sowing. According to this author the essential parts of the treatment were germination and freezing. According to Maximov (17, p. 432) Gratchef in 1874 found that by keeping the germinating seeds of artichokes at a temperature near 0° C. until the time of sowing he obtained plants which would produce a flower stalk while the controls remained in the vegetative stage. In 1911 (5) and 1918 (6) Gassner reported on the effect of low temperature germination on the subsequent development of winter and spring cereals. He found that seeds which germinated at 1° to 2° C. produced plants possessing very short vegetative periods, whereas seeds which germinated at higher temperatures remained in the vegetative stage considerably longer. He also observed that the develop-

ment of spring cereals was not affected by germination in the cold. From these results Gassner concluded that winter cereals differ from spring cereals in having a definite cold requirement which must be satisfied in order to obtain flowering.

Gassner's theory was contradicted by several investigators who on the basis of the results of their experiments claimed that winter cereals had no cold requirement. Murinov (19) subjected soaked seeds and seedlings at different stages of their development to freezing temperature and obtained no acceleration in reproduction of the plants from the treated seeds. Wacar (25) exposed germinating seeds of winter rye and winter wheat to a temperature of 2° to 6° C. for periods from 3 to 12 days, and also to temperatures below the freezing point for 30 days. Planting was done in February and all plants were kept in a warm greenhouse. He observed no difference in the time of heading of the treated plants as compared to that of the controls. Klages (8) planted Turkey Red wheat in October in a warm greenhouse. Part of the seedlings were exposed to a temperature below the freezing point for two weeks, whereas the others remained continuously in the greenhouse. Both lots headed simultaneously.

Dickson (3), on the other hand, confirmed Gassner's theory of cold requirement of winter cereals by reporting that Turkey Red and White Winter wheat kept at 5° C. all through the period of germination and up to the appearance of the first leaves gave 62 per cent heading, whereas the controls remained in the vegetative stage.

Maximov and Pojarkova (18) and Pojarkova (21) found no effect of germination temperature upon the heading time of winter wheat when sown in December to March, while if the grain was sown during March or April the seeds which germinated at 2° to 5° C. reached the reproductive stage much earlier than those which germinated at 15° to 20° C. Seeds sown in May headed only if germinated at the low temperature while those sown at later dates produced no heads even when germinated at the low temperature. The low temperature treatments used by Gassner, Maximov, and Wacar were of too short duration for the completion before sowing time of the processes determining the change from the vegetative to the reproductive stage. Therefore, these seeds produced plants which were able to head during the first year only if sown sufficiently early in the spring when the mean low temperature aided in bringing on the reproductive stage. A further improvement in the low temperature treatment of seeds was suggested by Tolmatschev (23) in a paper given at the U.S.S.R. Congress of Genetics, Plant and Animal Breeding held in January 1929. This investigator retarded the growth of seeds during the cold treatment by limiting the moisture in the germinating seeds. He thus succeeded in prolonging the low temperature treatment of winter wheat for two months and obtained plants which even with late spring sowing headed the first

At the same meeting Dolgushin and Lyssenko (4) reported that winter cereals could be brought to heads with late spring sowing by exposing the slightly germinated seed to low temperature (2° to 3° C.) treatments of definite duration before sowing. This process of "yarovization," that is, changing winter cereals into spring cereals, was described more fully by Lyssenko in later publications (11, 12, 13, 14, 24). Although other investigators had already succeeded in inducing winter cereals to head if sown in the spring after the critical date for the variety used, Lyssenko was the first to emphasize the practical importance of what so far were merely experimental results. According to this investigator the practical significance of the yarovization process consists in the production of earlier crops, in replanting winter-killed cereals in the spring with yarovized grain, in growing plants in regions where they do not usually reproduce, and in accelerating the work of plant breeders.

As a result of a number of experiments on the heading of winter cereals Lyssenko found that vegetative and reproductive development of the plant are not identical phenomena and that these different stages in the development of a plant require for completion definite external conditions. It follows that a most vigorous vegetative growth may continue indefinitely and the plant will not pass into the stage of heading unless the external conditions necessary for the passage from the vegetative to the reproductive stage have been fulfilled. Further, this transition from the vegetative to reproductive development of a plant may be brought on in a partially germinated seed which externally shows no sign of growth if the necessary conditions are supplied. The processes affecting the sexual reproduction of wheat, Lyssenko found, can occur not only in the well developed plant but also in seeds which have just commenced development but which have not yet broken the seed coat. The factor which induces the passage of winter cereals to the reproductive stage is low temperature in the presence of moisture and air. Lyssenko believes that each variety and species of winter cereal requires definite conditions for yarovization. These conditions are controlled by three factors: the moisture percentage of the seeds, the temperature of the treatment, and the duration of the treatment.

MATERIAL AND METHODS

Two varieties of winter wheat (*Triticum aestivum* L. var. Turkey Red and Leap's Prolific) were used in most of the studies. A few experiments were also carried out on four other varieties of winter wheat: Wisconsin Pedigree #2, Blackhull, Tenmarq, and Ilred; one variety of spring wheat (Blue Stem), and one variety of spring oats (*Avena sativa* L. var. Clydesdale).

The yarovization of seeds was accomplished according to the following method: the seeds were brought to definite moisture contents by soaking

weighed amounts of seeds in excess water for 12 hours at a temperature of 15° C. and then bringing them to the required weight by removing the excess of water. The moist seeds were kept at 15° C. for 24 hours more and then given a cold treatment of several weeks in a mechanical refrigerator. The plants were grown in greenhouses during the winter and in the field in the summer. Light conditions favorable for the heading of wheat were supplied during the winter months by the use of artificial light bringing the total day length to about 15 to 16 hours.

The effectiveness of the yarovization treatment under the conditions of the experiment was judged from two values, first the length of the vegetative period or the number of days from the date of planting to the date of appearance of the first heads, and second the percentage of plants which reached the reproductive stage on the 100th day after planting. The percentage was calculated on the basis of the number of living plants on that day.

RESULTS OF EXPERIMENTS

TEMPERATURE

Cold treatments were carried out at temperatures of 1°, 3°, 5° C., and at an alternating temperature of 3° to 5° C. From Table I, by comparing the results of groups of experiments in which temperature was the only varying factor, all other conditions being identical, it is seen that the cold treatments at 1° C. were slightly superior to treatments at 3° C. in their effectiveness in yarovizing winter wheat. In most cases the date of the appearance of the first heads was identical following storage at 1° and at 3° C. In a few experiments the 1° C. treatment gave better results than the 3° C. treatment, and in only one case the plants from the 3° C. treated seeds headed considerably earlier than those from the 1° C. lot. At temperatures of 3° C. and especially 5° C. it was difficult to prevent excessive germination and molding of the seeds. Temperatures above 3° C. were, consequently, found unsuitable for long treatments of winter wheat while 1° C. was concluded to be the most satisfactory temperature both as to efficiency of yarovization and as to keeping the treated seeds in good condition. For this reason the greater part of the experiments were carried out at a temperature of 1° C.

The results of a study of the effectiveness of temperatures below the freezing point also shown in Table I indicate that yarovization does not take place in the frozen seed. Under the conditions of the experiment apparently the Leap's Prolific seeds became yarovized at 1° C. in 48 days, a 24-day treatment at 1° C. produced a partial yarovization, and no further yarovization was produced by a similar interval at −12° C., these plants heading considerably later than those which received 48 days' treatment at 1° C. In the case of Turkey Red wheat the yarovization which

TABLE I

EFFECT OF TEMPERATURE OF TREATMENT ON THE YAROVIZATION OF WHEAT

Variety	Date planted	Cold treatment		Results	
		Temp. °C.	No. of days	Days for appearance of first heads	Heading on 100th day, %
T. R.	Apr. 28	1	50	68	37
" "	" "	3	"	No heading	0
" "	" "	3–5 altn.	"	No heading	0
" "	" "	1	55	65	50
" "	" "	3	"	No heading	0
" "	" "	5	"	No heading	0
" "	Mar. 23	1	67	86*	100*
" "	" "	3	"	86*	100*
L. P.	" "	1	"	81*	98*
" "	" "	3	"	82*	96*
T. R.	Apr. 6	1	81	72*	100*
" "	" "	3	"	72*	80*
L. P.	" "	1	"	77*	100*
" "	" "	3	"	72*	88*
T. R.	Aug. 26	1	48	110	0
" "	" "	1, −12 **	48	No heading	0
" "	" "	−12	48	No heading	0
L. P.	" "	1	48	83	70
" "	" "	1, −12 **	48	110	0
" "	" "	−12	48	No heading	0

* Average results from three separate experiments.
** 24 days at 1° followed by 24 days at −12°.

took place during 24 days of treatment at 1° C. was not sufficient to produce heading of the plants. The additional 24 days of freezing temperature had no stimulating effect on the heading. The results of these experiments indicate that yarovization does not take place at temperatures below 0° C., although during the process of yarovization freezing for 24 days at −12° C. does not kill the seeds. It follows that occasional lowering of the temperature below the freezing point during the cold treatment, while not injurious to the seeds, decreases the rate of yarovization.

STAGE OF GERMINATION OF SEEDS

After a 12-hour period of soaking and a 24-hour period of germination the plumules of most seeds were just breaking through the pericarp while in others there were no visible signs of germination. Throughout the yarovization treatment the seeds continued to germinate slowly. The rate at

which this germination proceeds depends to a certain extent upon the temperature, moisture, and aeration of the seeds. Low temperature decreases the rate of germination and freezing temperature stops the process entirely. High moisture content as a rule increases the rate of germination. If the amount of water supplied is larger than that which can be absorbed by the seeds, a thin film of moisture forms around each seed. This excess moisture either retards or completely stops germination.

It has been observed in these experiments that at the end of the low temperature treatment seeds which received different treatments varied

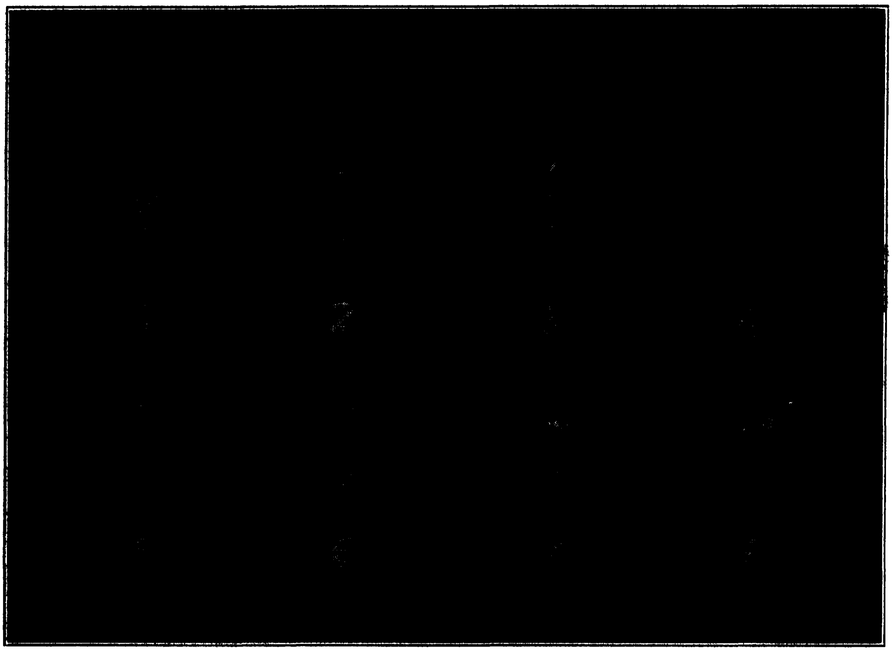


FIGURE 1. Different degrees of germination of Turkey Red wheat at the end of the low temperature treatment.

considerably in their stage of germination. The degrees of development shown in Figure 1 have been given arbitrary numbers 1, 2, 3, etc., and these numbers when used further indicate seeds in the stages of development shown in Figure 1.

There is some individual variation in the rate of germination of each seed and even seeds kept in identical conditions were often found to differ somewhat in their degree of germination at the end of the treatment.

In order to determine whether the condition of the seeds at the end of the cold treatment and consequently also the rate of germination of the seeds during the treatment had any effect on the subsequent behavior of

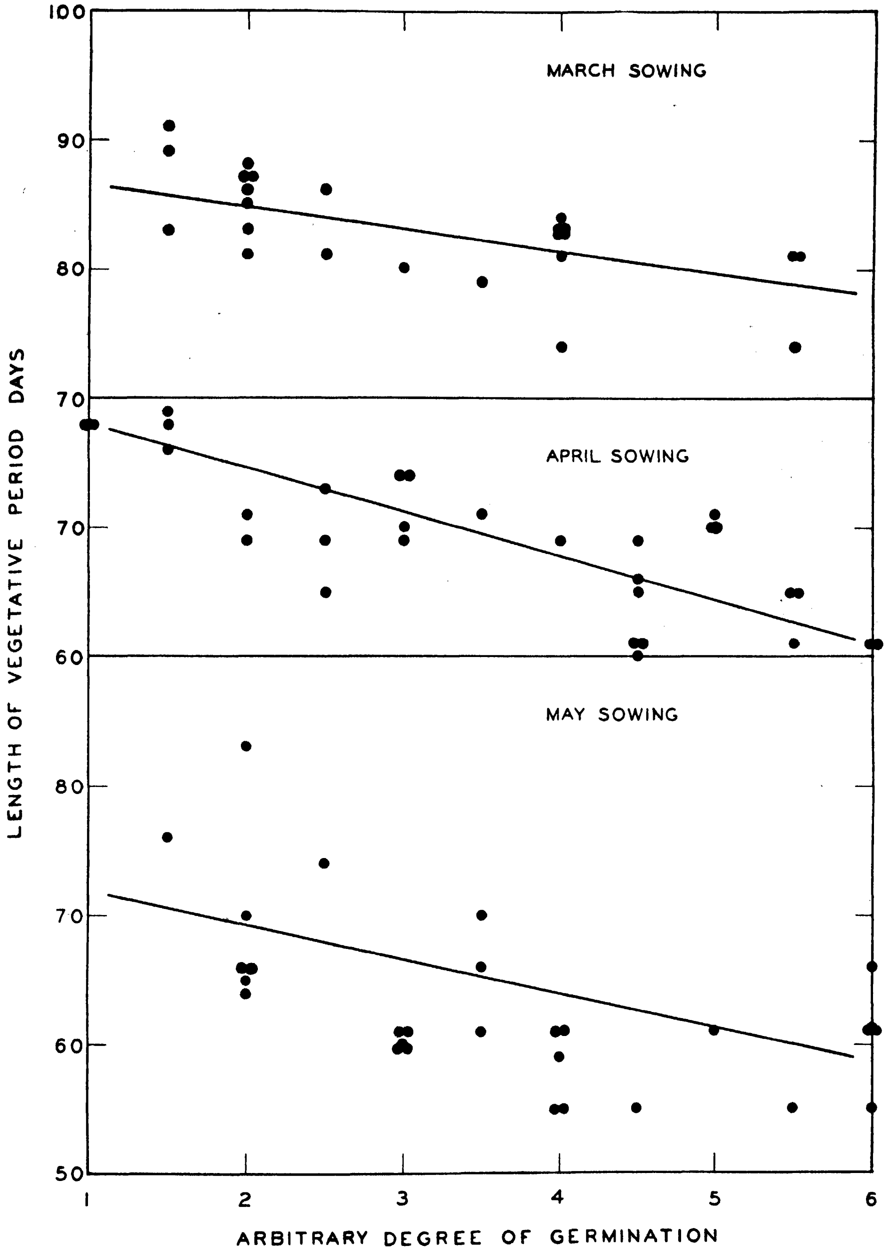


FIGURE 2. Relation between the degree of germination of the seeds at the end of the low temperature treatment and the length of the vegetative period of the plants (see Fig. 1).

the plant, the following tests were carried out. The seeds or seedlings which had received identical treatments and had been exposed to low temperature in the same state of germination were divided into several groups on the basis of the degree of their germination at the end of the cold period. The different groups were planted separately and kept under identical conditions. The only variable factor in these groups was the rate of development of the seeds and, therefore, any difference in the effectiveness of the yarovization could be attributed only to this factor. The results of these experiments and other experiments performed with seeds which were all in the same state of germination at the beginning of the low temperature treatment and were kept at varying adequate yarovization conditions are shown in Figure 2. In these graphs the different stages of the germination of adequately yarovized seeds (Fig. 1) are plotted on the abscissa and the corresponding values for the length of the vegetative growth of the plants on the ordinate. By applying the method of least squares the equations for the lines representing the relationship between the two variables were calculated. The slope of the lines obtained proves that there is a correlation between the state of germination of the seeds at the end of the low temperature treatment (which under the conditions of the experiment corresponds to the rate of germination during the treatment) and the degree of yarovization of the corresponding plants. The length of the vegetative period of the plant decreases with the increase in the rate of germination of the seed.

In several experiments the seeds were allowed to germinate for 48 hours after the 12-hour soaking period. These seeds were in a more advanced degree of germination (#4 and #5, Fig. 1) at the beginning of the low temperature treatment than the seeds which received the regular 24-hour germination period. When exposed to identical cold treatments the seeds of both groups produced plants which did not differ in their degree of yarovization.

The results of these experiments suggest that regardless of the degree of germination of the seeds at the beginning of the cold treatment, seeds which are undergoing a more active process of development during the cold treatment produce plants of a higher degree of yarovization as compared with plants from seeds which germinate very little during the treatment.

MOISTURE CONTENT OF SEEDS

Moisture required for yarovization. Experiments were performed with the moisture content of seeds varying from 40 to 80 per cent (on the basis of the dry weight before germination). According to Lyssenko's instructions for yarovization (24) the seeds are brought to a definite moisture percentage just once before the beginning of the cold treatment. It is

evident that a certain amount of drying occurs during the treatment. Consequently in this case yarovization proceeds at a gradually decreasing moisture content. In order to have yarovization proceed at a constant moisture percentage of the seed it is necessary to adjust the moisture percentage of the seed throughout the whole period of cold treatment. This was done by weighing the seeds periodically throughout the treatment and adding water whenever a decrease in weight indicated that a loss of moisture had occurred.

TABLE II

EFFECT OF CONSTANT (C) AND GRADUALLY DECREASING (GD) MOISTURE PERCENTAGES DURING THE COLD TREATMENT ON THE YAROVIZATION OF WHEAT (TURKEY RED)

Time of planting	Moisture, per cent		Length of treatment, days	Results	
	Initial	Throughout the exp.		Days for appearance of first heads	Heading on 100th day, %
April	40	GD	55	No heading	0
"	40	C	55	" "	0
"	50	GD	55	No heading	0
"	50	C	55	63	70
"	55	GD	55	No heading	0
"	55	C	55	68	100
"	60	GD	55	75	12
"	60	C	55	73	75
"	80	GD	55	65	100
"	80	C	55	71	85
July	50	GD	111	No heading	0
"	50	GD*	111	118	0
"	50	GD	118	No heading	0
"	50	GD**	118	92	44

* 50 per cent for last 2 weeks of treatment.
** 50 per cent for last 3 weeks of treatment.

The difference in the results obtained by using these two methods can be seen in Table II. Under the conditions stated in this table, initial moisture contents of 50 and 55 per cent which gradually decreased were not sufficient to produce yarovization. The same moisture contents were satisfactory when kept at these values constantly. At higher moisture contents the difference was less striking; at 60 per cent moisture yarovization proceeded regardless of drying. When drying took place, however, a lower percentage of heading occurred. The effect of the adjustment of the moisture content can also be seen from the data presented in Table II (July plantings). In these experiments low temperature treatments of 16 and 18 weeks at an initial moisture content of 50 per cent did not produce

any noticeable yarovization. If the moisture content of the same seeds was brought back and kept at its initial value during the last two or three weeks of treatment, other conditions being identical, yarovization took place. Evidently in determining the moisture necessary for the treatment it is essential to differentiate between the initial moisture content and the constant moisture content of seeds during yarovization.

Table III indicates the effect of different moisture contents of the seed on the yarovization of wheat. The figures representing the results of the

TABLE III

EFFECT OF THE MOISTURE CONTENT OF SEEDS DURING THE COLD TREATMENT ON THE YAROVIZATION OF WHEAT

Moisture, % (constant)	Date planted	Average results					
		Turkey Red			Leap's Prolific		
		Vegetative period, days	Heading on 100th day, %	No. of experiments	Days for appearance of first heads	Heading on 100th day, %	No. of experiments
40	End of March	93*	80	1	97*	60	1
45		90	83	2			
50		86	96	2	82	93	5
55		85	96	2	80	93	5
60		84	98	2	80	93	5
70		86	81	1	85	93	2
80					94	78	1
45	April	78	74	3			
50		61	100	3	67	93	7
55		63	100	5	67	87	7
60		61	100	4	67	93	7
70		67	88	4	73	81	2
80		71**	100	3	76	46	1
50	May	62	90	2			
55		59	100	2			
60		57	100	2			
70		61**	90	2			

* Untreated wheat started heading on same date.
** No heading in several cases.

treatments are the average values obtained from several sets of experiments performed at different adequate conditions of temperature and length of treatment. The results (Tables II and III) indicate that a moisture content maintained constantly at 40 per cent was too low to keep the seeds in an active stage of germination and to produce yarovization. Constant moisture contents of 50 per cent and up to 70 per cent proved to have an adequate stimulating effect, moisture contents of 60, 55, and 50 per cent giving the best results. A further increase in the moisture content

to 70 per cent produced a slight, and to 80 per cent a marked decrease in the degree of yarovization. Treatments given at gradually decreasing moisture contents of the seeds required a minimum initial moisture of 60 per cent. In these cases moisture contents of 70 and 80 per cent were found to produce adequate yarovization.

It is evident that under the conditions of gradually decreasing moisture the effectiveness of the treatment depends greatly upon the rate of drying of the seeds. Yarovization will take place only if the rate of drying is such that the seeds remain at the effective moisture contents (between 50 and 70 per cent) for a period long enough for the completion of the necessary processes.

Effect of drying the yarovized seed. Several lots of completely yarovized seeds were air-dried and kept for four weeks at temperatures of 1° C. and 15° C. The average results of these experiments indicate that the dried seeds both from the 1° C. and 15° C. sets produced plants which either headed later than the plants from the moist seeds, or remained in the vegetative stage. Drying, especially at the higher temperature, produced also a very marked decrease in the percentage germination of the planted yarovized seeds.

Apparently both drying and temperatures above those required to produce yarovization reduce the properties developed by the seeds during the process of yarovization.

LENGTH OF YAROVIZATION TREATMENT

Shortest adequate low temperature treatments. The effect produced by different durations of the cold treatment of the seeds on the rate of the passing of the plants into the reproductive stage is given in Table IV. The figures representing the results of the treatments are the average values obtained from several (4 ± 2) sets of experiments performed at different temperatures and moisture percentages found to be satisfactory for the completion of the process of yarovization.

The number of days of natural yarovization, that is, the number of days of low temperature treatment received by the plants after sowing (columns 3 and 10) was obtained by calculating from the data given by the U. S. Weather Bureau maps the number of days, or fractions of days, during the period of growth of the plant at which the temperature was below 10° C. (According to Lyssenko, yarovization takes place at a temperature as high as 10° C.)

Table IV indicates that the length of the cold treatment necessary to produce adequate yarovization decreases with the increasing number of days of low temperature following sowing. Plants kept continuously at warm temperature required a minimum cold treatment of about 68 days; while for seeds sown at the end of March and consequently exposed to

TABLE IV
EFFECT OF LENGTH OF TREATMENT ON THE YAROVIZATION OF WHEAT

Var.	Days cold treated: Before planting	Days cold treated: After planting	Date planted	Average results: Days for 1st head	Average results: % heads on 100th day	Col. 2 + Col. 5, days
T. R.	57	0	5/18	—	0	—
	68	0	"	62	100	130
	92	0	"	63	75	155
L. P.	57	0	"	—	0	—
	68	0	"	62	100	130
	92	0	"	60	100	152
T. R.	49	5	5/4	—	0	—
	62	5	"	60	100	122
	78	5	"	56	100	134
L. P.	49	5	"	—	0	—
	62	5	"	60	100	122
	78	5	"	56	100	134
T. R.	42	8	4/27	—	0	—
	56	8	"	64	100	120
	71	8	"	62	100	133
	86	8	"	62	100	148
L. P.	42	8	"	—	0	—
	56	8	"	68	100	124
	71	8	"	70	100	141
	86	8	"	62	94	148
T. R.	35	10	4/20	—	0	—
	51	10	"	69	94	120
	64	10	"	61	100	125
	70	10	"	63	100	142
L. P.	35	10	"	—	0	—
	51	10	"	75	85	126
	64	10	"	64	95	128
	70	10	"	63	96	142
T. R.	28	14	4/15	—	0	—
	42	14	"	69	100	111

Var.	Days cold treated: Before planting	Days cold treated: After planting	Date planted	Average results: Days for first head	Average results: % heads on 100th day	Col. 9 + Col. 12, days
T. R.	0	16	4/13	156	0	156
L. P.	0	16	"	156	0	156
T. R.	44	16	"	76	88	120
	57	16	"	76	100	133
	72	16	"	76	100	148
L. P.	44	16	"	73	78	117
	57	16	"	70	87	127
	72	16	"	66	94	138
T. R.	34	22	4/6	87	90	121
	50	22	"	78	100	128
	65	22	"	88	100	153
	81	22	"	76	94	157
L. P.	34	22	"	89	76	123
	50	22	"	77	83	127
	65	22	"	72	98	137
	81	22	"	76	90	157
T. R.	27	30	3/30	92	80	119
	43	30	"	89	100	132
	58	30	"	82	100	140
	0	30	"	116	100	116
L. P.	27	30	"	83	79	110
	43	30	"	82	93	125
	58	30	"	76	94	134
	0	30	"	116	0	116
T. R.	51	37	3/23	85	91	136
	67	37	"	87	100	154
	0	37		93	100	93
L. P.	51	37	"	82	97	133
	67	37	"	81	94	148
	0	37	"	97	50	97

30 days of cold weather a preliminary cold treatment of 27 days was sufficient. This was the shortest treatment used in this experiment and there is the possibility that a treatment even shorter than 27 days might give positive results under these conditions.

From Table IV by adding the corresponding figures of columns 2 and 3 and columns 9 and 10 one can see that the minimum effective value for the total number of days of cold treatment received by the plant both before and after sowing lies roughly between 56 and 68 days. There seems to be a tendency for the total minimum number of days of cold treatment necessary to produce definite yarovization effects to decrease slightly with the increase in the number of days of natural yarovization. The difference between the shortest effective cold treatment and the longest non-effective treatment was usually about 14 days; moreover, the shortest treatments tried were frequently found to be effective. This indicates that the minimum effective dose has been determined within too wide limits to allow these conclusions to be drawn from small changes in its values. On the other hand, 57 and 54 days' cold treatments for the May 18 and May 4 sowing respectively were inadequate while complete yarovization was produced by 56 and 57 days' treatments at earlier sowings (March 30 and April 6) for both Turkey Red and Leap's Prolific wheat. Better results were also obtained by a 56-day treatment at both 60 and 80 per cent moisture of Blackhull and at 80 per cent moisture of Ilred, Tenmarq, and Wisconsin Pedigree #2 varieties sown early in the spring as compared to the results of similar treatments given to plants grown continuously in the warm temperature (Table VI). These facts seem to give strong indications that the total cold treatment necessary to bring winter wheat into the reproductive stage is shorter in case of early spring sowings than it is for late sowing in which all the required low temperature is supplied during the treatment of the seeds.

This phenomenon is in agreement with the previous observation that yarovization proceeds more efficiently under conditions which are favorable for the active development of the plant. Such conditions exist usually during the natural yarovization where the seed reaches advanced stages of germination while undergoing the cold treatment.

Effect of treatments of different durations on the yarovization of wheat. As a result of a series of experiments carried out in greenhouses it was observed that winter wheat varieties, such as Leap's Prolific and Turkey Red, grown continuously in the warm greenhouse (16° to 22° C.) under either short day for the first two weeks followed by a long day, or continuous long day have a vegetative period of about 150 days. Cold treatments may produce shortening of the vegetative period to less than half of this time. From Figure 3 it is apparent that for treatments varying from 30 to 60 days, other conditions being identical, the length of the vegetative

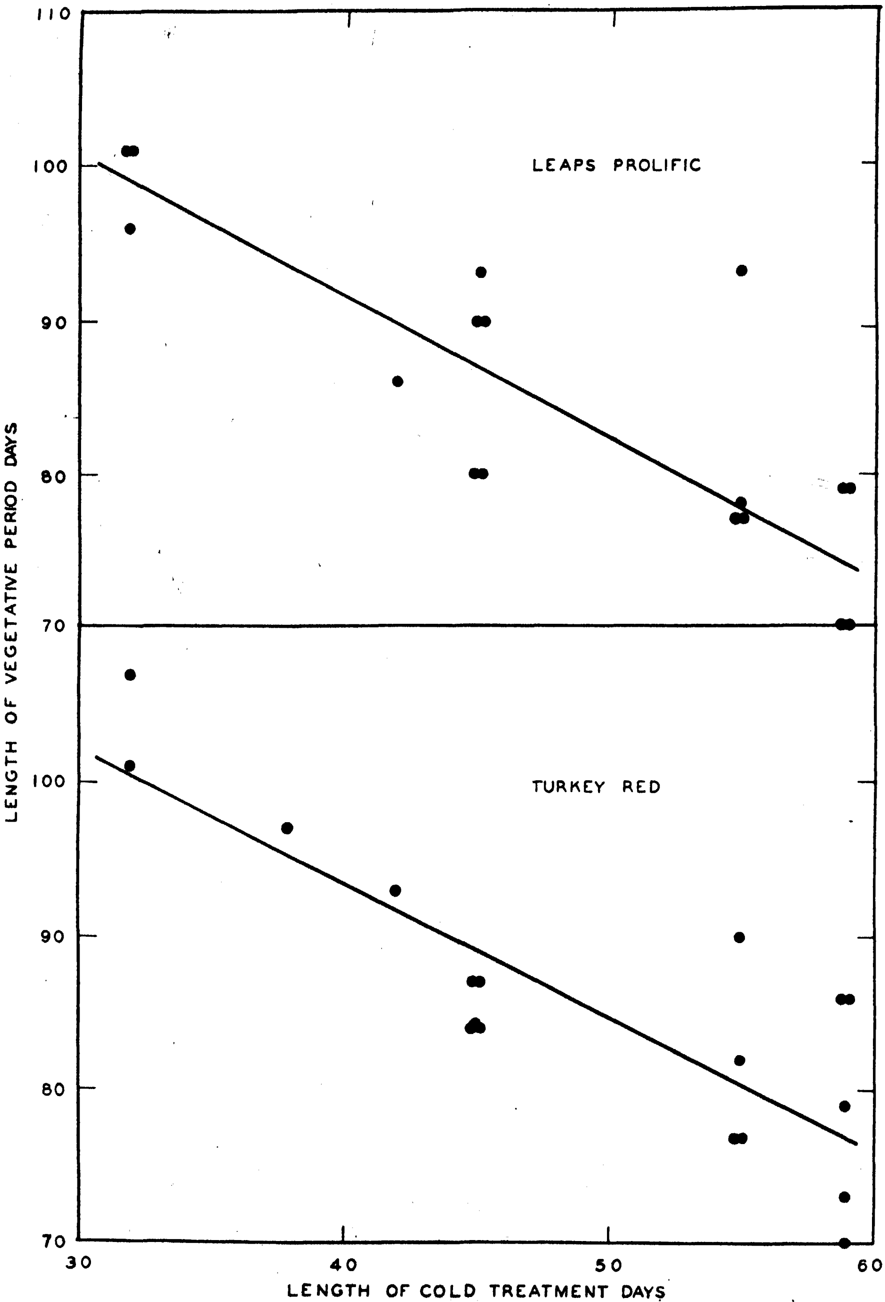


FIGURE 3. Relation between the duration of low temperature treatments and the length of vegetative periods.

period decreases regularly with the length of the low temperature treatment. Cold treatments between 60 to 70 days produce complete yarovization. When a seed has reached the stage of complete yarovization further increase of the cold treatment produces very little or no shortening of the vegetative phase of the plant.

With late spring sowing of partly yarovized plants in field experiments, the plants either remained in the vegetative stage or produced a little irregular heading. As already stated, untreated Turkey Red and Leap's Prolific headed when grown in the greenhouse kept at 16° to 22° C. showing that low temperature is not absolutely necessary for the passing of winter wheat into the reproductive stage, but only shortens the vegetative period of the plants. The inability of inadequately yarovized and untreated winter wheat sown late in the spring to head during the first summer is due to the fact that in the absence of the stimulating effect of cold the plants vegetate for about five months. By the end of that period the temperature and light conditions become unfavorable for heading and the plants remain in the vegetative stage.

It has been observed that prolongation of the low temperature treatment beyond the minimum duration required for the completion of yarovization processes produces in most cases a very slight shortening of the vegetative period. It has also been found that treatments as long as 105, 122, 137, and 175 days produced highly yarovized seeds as seen in Turkey Red and Leap's Prolific wheat yarovized for 137 days (Fig. 4). It follows from these observations that excessively prolonged cold treatments do not destroy the yarovization acquired by the seed.

EFFECT OF YAROVIZATION ON THE LENGTH OF THE TOTAL PERIOD OF VEGETATIVE DEVELOPMENT

It is of interest to find out whether the acceleration produced in the development of the plant by the cold treatment consists in the shortening only of its vegetative growth (from the date of planting to the date of heading), or whether it shortens the whole life of the plant from the beginning of the treatment to the day of heading. The length of this time is evidently composed of two periods: the time of yarovization treatment, and the time of vegetative growth. By summing up these values in the experiments reported in Table IV the figures represented in columns 7 and 14 of this table are obtained. From these figures it is evident that the total life of the plant from the time of the soaking of the seed to the time of heading is shortened by satisfactory yarovization treatments. Taking 150 days as the number of days necessary for the completion of the vegetative growth by a non-yarovized plant, the acceleration produced by the shortest adequate yarovization treatment is found to vary from 20 to 40 days. A prolongation of the low temperature treatment beyond the time

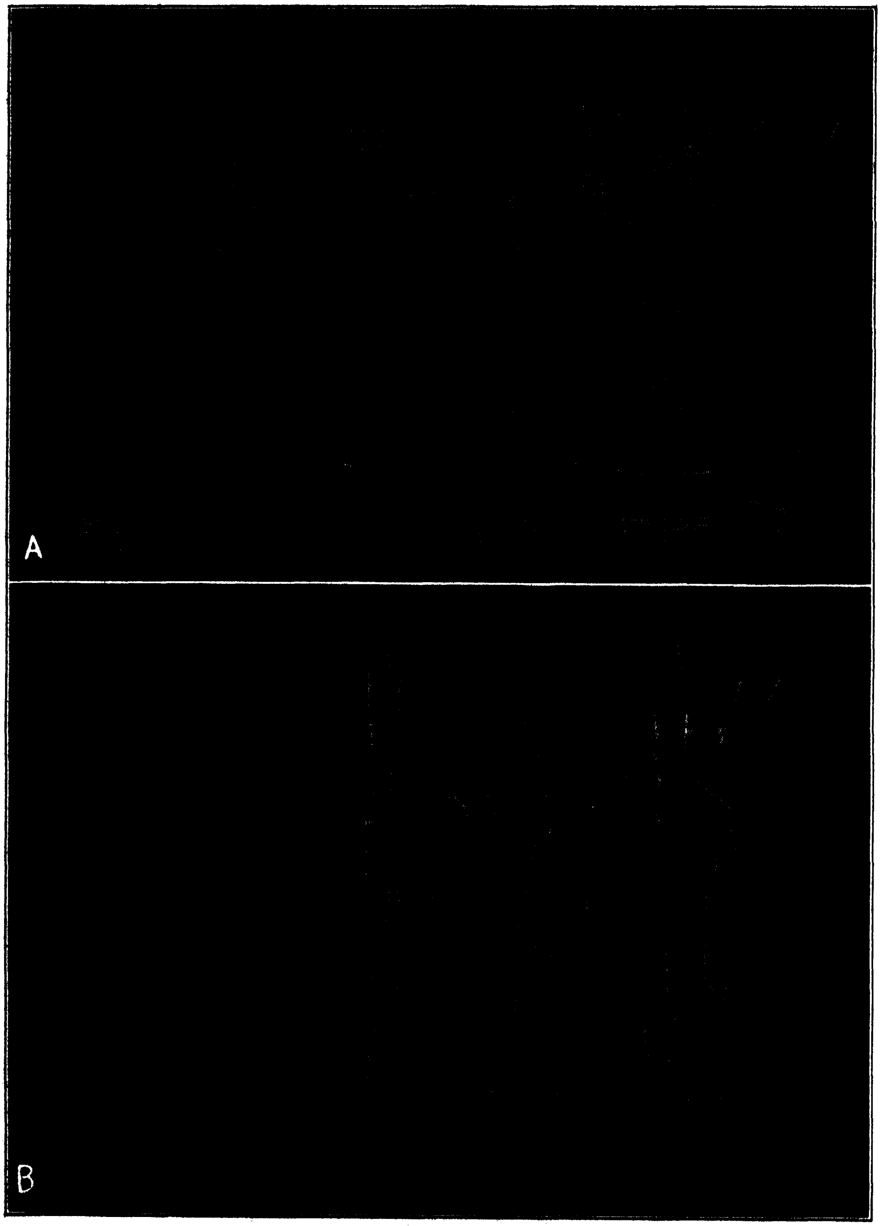


FIGURE 4. Wheat yarovized for 137 days. A. Turkey Red. B. Leap's Prolific. Control at extreme left.

necessary for complete yarovization produces an increase in the length of the period from soaking to heading. Evidently the changes occurring in the germinating seed under the influence of low temperature which stimulate the heading of the plant are completed after a certain time. Further yarovization treatment produces very little or no development in the plant, the seeds remaining unchanged until sowing time, when the second period of the development of the plant starts under the influence of warm temperature and light.

FIELD CONDITIONS

Effect of date of planting on the length of the vegetative period. It has been shown earlier in this paper that in field experiments winter wheat sown early in the spring, and consequently exposed to natural yarovization temperatures, passed into the reproductive stage following very short pre-sowing treatments, while wheat sown later in the summer required a considerably longer preliminary cold treatment.

The date of planting has also an effect on the rate of passing into the reproductive stage of the completely yarovized plant. It has been observed (Table IV) that the length of the vegetative period of completely yarovized plants varied from 55 to 64 days for May plantings, and from 81 to 94 days for March plantings. Apparently the conditions of long day and warm temperature supplied to the yarovized plant following late sowing are favorable for the rapid heading of winter wheat.

Soil fertility. In several experiments yarovized seeds were sown in poor soil, which was unsatisfactory for the normal growth of wheat. Among the plants which survived under these conditions some reached a height of only three to five inches. These small plants started heading at about the same time as the similarly treated wheat grown in good soil. Evidently yarovization stimulates head formation in poorly developed weak plants to the same extent as it does in normal plants.

GERMINATION OF THE PLANTED YAROVIZED SEEDS

The effect of the yarovization treatment on the percentage germination (seedling emergence from soil) of wheat is shown in Table V. The figures in column 3 represent the decrease in the per cent germination of the completely yarovized wheat as compared to that of the untreated wheat sown on the same date. Each figure is the average of a number of experiments (column 4) carried out at adequate yarovization conditions.

The results indicate that yarovization treatment does not affect the per cent germination of Turkey Red wheat planted in pots. On the average the percentage germination gave identical values for treated and control seeds. Yarovized Turkey Red wheat planted in the field invariably had a lower percentage germination than the corresponding control. The de-

crease in the percentage germination varied considerably in the different cases. The average decrease calculated from 97 experiments was equal to 11 per cent. Apparently wheat loses some of its resistance to unfavorable external conditions during the process of yarovization. The treated seeds were able to germinate normally in the optimum soil and moisture con-

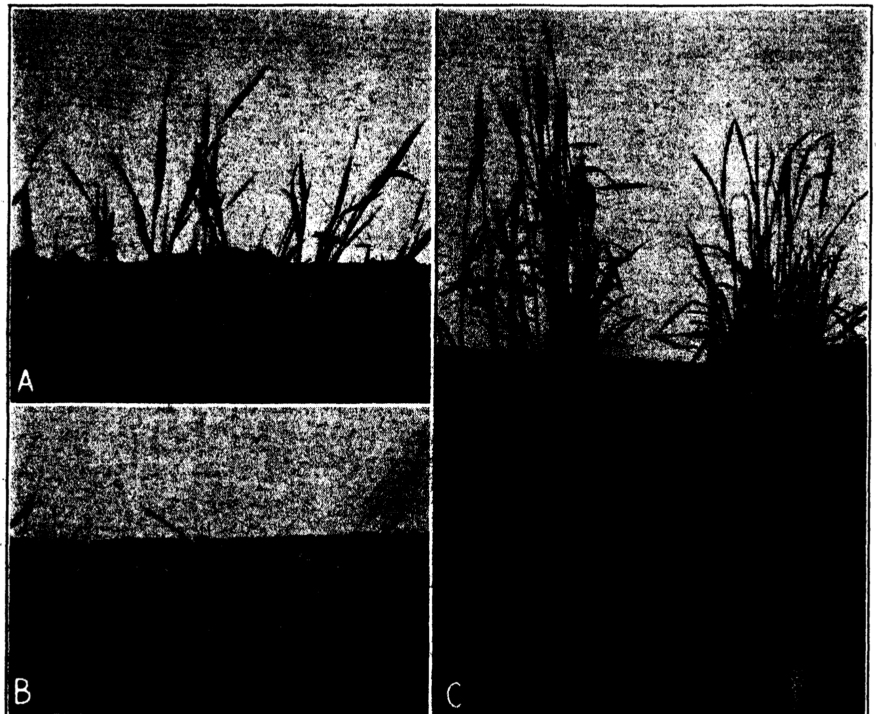


FIGURE 5. Yarovization of Turkey Red wheat. A. Yarovized Turkey Red one month after germination. B. Corresponding control. C. Left, yarovized Turkey Red two months after germination; right, corresponding control.

TABLE V

DECREASE IN PER CENT GERMINATION OF YAROVIZED WHEAT AS COMPARED TO UNTREATED WHEAT

Variety	Grown in	Decrease in % germ.	Number of experiments averaged
Turkey Red	Pots	0	52
Turkey Red	Field	11	97
Leap's Prolific	Pots	5	25
Leap's Prolific	Field	20	67
Blackhull	Field	8	4
Ilred	Field	10	4
Tenmarq	Field	8	4
Wisconsin Pedigree #2	Field	7	4

ditions supplied in the pot experiments, while they showed a decreased germination in the less favorable field conditions.

Results of a similar character were observed in experiments with Leap's Prolific wheat. The decreases in the per cent germination of the treated seeds of this variety were 5 and 20 per cent for pot and field experiments respectively. It was generally observed that Leap's Prolific seeds were

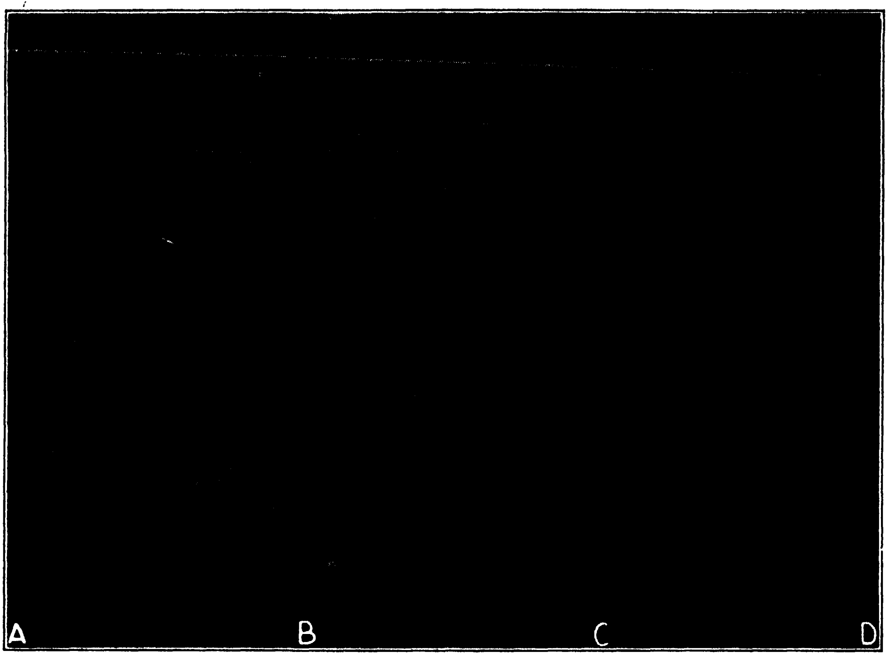


FIGURE 6. Yarovization of Turkey Red. A. Control. B and C. Partly yarovized wheat. D. Completely yarovized wheat.

more susceptible to molding and rotting during the process of yarovization than were the seeds of the Turkey Red wheat variety. A few experiments carried out with some other varieties of winter wheat (Blackhull, Ilred, Tenmarq, and Wisconsin Pedigree #2) suggest that in these seeds also yarovization treatment produces a decrease in germination.

GROWTH HABIT OF YAROVIZED WHEAT

The effect of the yarovization treatment on the growth habit of wheat could be observed soon after germination. While control plants showed excessive tillering, the treated plants were more erect. The appearance of a set of yarovized plants (sown on March 23) and the corresponding controls one and two months after germination is shown in Figure 5 A, B, C.

In late spring sowing the completely yarovized wheat showed very little vegetative growth (Fig. 6 D). Plants which received a less effective

yarovization treatment produced much vegetation before stem elongation started (Fig. 6 B, C). The untreated winter wheat remained in the tillering stage (Fig. 6 A). Untreated Turkey Red and Leap's Prolific wheat sown on March 23 were heading by the end of June. Later plantings produced very few or no heads. For the existing climatic conditions the critical date for Turkey Red and Leap's Prolific wheat can be considered to be around March 23.

YAROVIZATION OF DIFFERENT VARIETIES

From the results presented in Tables I, III, and IV, it is evident that the two varieties of winter wheat, Turkey Red and Leap's Prolific, do not differ in the conditions required for their yarovization. A few experiments carried out with four other varieties of winter wheat (Table VI) indicate that Ilred, Wisconsin Pedigree #2, and Tenmarq respond to shorter treatments, while Blackhull requires a treatment as long as Turkey Red wheat.

TABLE VI

YAROVIZATION OF BLACKHULL, ILRED, TENMARQ, AND WISCONSIN PEDIGREE #2

Variety	Days of treatment			Date planted	Results obtained when seeds kept at			
					60% moist.		80% moist.	
	Cold treatment	Natural cold treatment	Total		Days for appearance of first heads	Heading on 100th day, %	Days for appearance of first heads	Heading on 100th day, %
Blackhull	55	0	55	Jan. 3*	No heading	No heading	No heading	No heading
	76	0	76	May 18	63	80	63	100
	62	5	67	May 4	58	100	61	82
	55	8	63	Apr. 27	62	92	74	42
	40	16	56	Apr. 13	72	90	81	56
Ilred	55	0	55	Jan. 3*	78	100	No heading	No heading
	76	0	76	May 18	60	100	60	100
	62	5	67	May 4	58	100	61	100
	55	8	63	Apr. 27	67	88	70	100
	40	16	56	Apr. 13	72	75	76	89
Tenmarq	55	0	55	Jan. 3*	58	100	No heading	No heading
	76	0	76	May 18	60	100	80	37
	62	5	67	May 4	60	92	99	5
	55	8	63	Apr. 27	67	83	100	10
	40	16	56	Apr. 13	76	100	90	70
Wisconsin Pedigree #2	55	0	55	Jan. 3*	58	100	No heading	No heading
	76	0	76	May 18	53	100	63	75
	62	5	67	May 4	60	100	74	85
	55	8	63	Apr. 27	67	100	83	30
	40	16	56	Apr. 13	76	90	81	43

* Grown in greenhouse.

Two kinds of spring cereals, Blue Stem wheat and Clydesdale oats, treated at different temperatures (1°, 3°, and 5° C.) and moisture contents (45, 50, and 55 per cent) for periods from 14 to 58 days invariably failed to respond to the yarovization treatment. In all cases the plants from the treated seeds passed to the reproductive stage on the same date as the corresponding controls. Evidently low temperature treatment does not shorten the vegetative period of these varieties of spring cereals.

DISCUSSION

It has been repeatedly observed in this work that winter wheat grown continuously in the warm temperatures produces heads. Consequently, winter wheat does not necessarily require low temperature in order to flower. The rôle of low temperature, whether supplied to the plant or to the slightly germinated seed, consists in shortening the vegetative period of the plant, thus enabling it to pass into the reproductive stage early enough in the summer while the temperature and light conditions are favorable for flowering.

The failure of many attempts of the early investigations to stimulate the heading of winter cereals by low temperature treatment applied to the seedlings was apparently due to the fact that too short cold treatments were used by some investigators (18, 25) while others subjected the seedlings to unsuitable temperature conditions (8, 19, 25).

In this work the results of 688 experiments carried out at varying conditions both of pre-sowing treatment of the seeds and of the subsequent growth of the plant indicate that yarovization can proceed only if the seed continuously undergoes a slow process of germination during the low temperature treatment. Any condition which checks further germination of the seed such as freezing, drying, or excessive moisture prevents the process of yarovization. Other investigators (18, 24) have also reported that yarovization does not proceed in frozen seeds, while Klippart (9) claimed that freezing was essential for conversion of winter wheat into spring wheat.

The shortest cold treatment necessary for the completion of yarovization processes differs for the different varieties and depends also upon the time of sowing and later climatic conditions, each day of low temperature experienced in early spring in the field producing an additional natural yarovization and consequently decreasing the number of days of artificial yarovization required by the plant. Cold treatments of insufficient duration produce partly yarovized plants, each length of treatment of the seed corresponding to a definite length of vegetative period of the plant subsequently grown under identical light and temperature conditions. The observation that seeds can be brought to different stages of yarovization indicates that the changes produced in the seed under the influence of the

cold pre-sowing treatment proceed gradually. After a treatment of a requisite length of time the seeds reach a phase when further treatment produces very little or no effect.

Very long treatments produce highly yarovized plants. This suggests that excessively prolonged cold treatments do not destroy the properties acquired by the seed during the yarovization treatment. This conclusion is in disagreement with the theory of reversibility of yarovization brought out by Cerling and Cepikova (2) and Gavrilova (7). These investigators claim that the process of yarovization proceeds only for a certain period under the influence of low temperature. Further treatment produces a reversed process. They believe that by using a treatment 50 per cent to 100 per cent longer than that required for the completion of yarovization it is possible to bring the seeds back to a condition which will not differ from the checks in the behavior after sowing. It was observed that the effect of yarovization could be decreased or nullified by drying the yarovized seed and keeping it for several weeks at a warm temperature. This reverse process starts when the conditions of the seed are changed from high moisture content and low temperature to low moisture content and higher temperature. Consequently, the yarovization process is not reversible as long as the seed is kept in the conditions required for yarovization.

The length of the vegetative phase of the completely yarovized plant depends upon environmental conditions under which the plants are grown, long day and warm temperature stimulating early heading at that stage of the development of the plant. These results are in agreement with Lyssenko's theory that in order to reach the reproductive phase the plant has to pass through two different stages of development. The first of these stages of development is stimulated by low temperature, while the second requires warm temperature and long day.

In these experiments no acceleration of the heading of a spring wheat, variety Blue Stem, could be produced by a cold treatment. It has been repeatedly reported in the literature (1, 16, 22) that cold treatment has no effect on the time of heading of spring wheat. On the other hand Lyssenko and other investigators (10, 13, 14) reported that yarovization induces early heading in spring wheat. In all cases an acceleration of only a few days has been reported by these investigators and it seems probable that this acceleration is due to earlier seedling emergence of partially germinated treated seeds as compared to that of the control.

Yarovization of winter wheat by cold treatment carried out in refrigerators has been successfully accomplished by many investigators in this field (1, 15, 20). Whether yarovization by natural cold treatment is possible evidently depends upon the existing climatic conditions. The fact that yarovization proceeds successfully at low temperatures (1° to 3° C.)

while higher temperatures induce over-germination and mold during the long period required for yarovization of winter wheat suggests that the pre-sowing treatment can be carried out in barns as suggested by Lyssenko (24) only in climates where the temperature does not rise above zero through the period necessary for the pre-sowing treatment.

SUMMARY

1. Temperatures of 1° to 3° C. were satisfactory in producing yarovization of Turkey Red and Leap's Prolific wheat. At temperatures above 3° C. molding and excessive germination took place.

2. Yarovization did not proceed at freezing temperatures, although freezing during the process did not kill the seeds.

3. Seeds which germinated actively in refrigerator treatment produced plants of a higher degree of yarovization.

4. Yarovization proceeded at any moisture content which was suitable for sustaining the seeds in an active state of germination. For the varieties used moistures of 50 to 70 per cent maintained continuously were found adequate. An initial moisture of not less than 60 per cent was required if the moisture content of the seeds was not further adjusted during the cold treatment.

5. Drying the yarovized seeds and exposing them to warm temperatures decreased or nullified the yarovization already produced.

6. The duration of the low temperature treatments necessary to produce yarovization was found to be 9 to 10 weeks for Turkey Red, Leap's Prolific, and Blackhull wheat, and about 8 weeks for Ilred, Wisconsin Pedigree #2, and Tenmarq. These varieties required a shorter treatment if sown early and exposed to the natural low temperatures of spring.

7. Prolonged low temperature treatments did not nullify the yarovization properties.

8. With a long day and warm temperature yarovized seed sown in May headed in 56 days while that sown early in the spring headed in approximately 80 days.

9. Turkey Red and Leap's Prolific wheat not yarovized but grown continuously at a temperature of 16° to 22° C. and a day length of 15 to 16 hours headed in about 150 days after sowing. In the completely yarovized plant the total length of time from the beginning of the cold treatment to the end of the vegetative period was equal to 110 to 120 days under most favorable conditions.

10. Yarovization treatment produced a decrease in the percentage germination of wheat sown in the field.

11. Low temperature treatments did not shorten the vegetative period of the spring cereals: Blue stem wheat and Clydesdale oats.

LITERATURE CITED

1. BELL, G. H. D. Preliminary experiments on vernalization. Jour. Agric. Sci. **25**: 245–257. 1935.
2. CERLING, V., and A. CEPIKOVA. On the question of the type of the process of yarovization. II. Compt. Rend. Acad. Sci. URSS **1934**(3): 542–543. 1934.
3. DICKSON, JAMES G. The relation of plant physiology and chemistry to the study of disease resistance in plants. Jour. Amer. Soc. Agron. **17**: 676–695. 1925.
4. DOLGUSHIN, D. A., and T. D. LYSSENKO. To the question of the substance of winter-crops. Proc. U.S.S.R. Congr. Genet., Plant and Animal Breed. Leningrad, 1929. **3**: 189–199. 1929.
5. GASSNER, GUSTAV. Beobachtungen und Versuche über den Anbau und die Entwicklung von Getreidepflanzen im subtropischen Klima. Jahresber. Verein. f. Angew. Bot. **8**: 95–163. 1911.
6. ——— Beitrage zur physiologischen Charakteristik sommer- und winter-annueller Gewachse, insbesondere der Getreidepflanzen. Zeitschr. Bot. **10**: 417–480. 1918.
7. GAVRILOVA, M. On the reversibility of the vernalization process. Compt. Rend. Acad. Sci. URSS **1935**(1)· 562–563. 1935.
8. KLAGES, K. H Metrical attributes and the physiology of hardy varieties of winter wheat. Jour. Amer. Soc. Agron. **18**: 529–566. 1926.
9. KLIPPART, JOHN M. An essay on the origin, growth, diseases, varieties, etc. of the wheat plant. *In* Ohio State Bd. of Agric. Ann. Rept. **1857**: 562–816. 1858.
10. LOWIG, E. Jarowisationsversuche. Deutsch. Landw Presse **61**: 409–410. 1934.
11. LYSSENKO, T. D. K voprosu o regulirovanii dliny vegetatsionnogo perioda S.-Kh. rastenii. Biull. Iarovizatsii. **1**: 5–13. 1932.
12. ——— Iarovizatsii͡a sel'sko-khozi͡aĭstvennykh rastenii. Biull. Iarovizatsii **1**: 14–29. 1932.
13. ——— Predvaritel'noe soobshchenie o i͡arovizirovannykh posevakh pshenits v sovkhozakh i kolkhozakh v 1932 g. Biull. Iarovizatsii **2–3**: 3–16. 1932.
14. LYSSENKO, T. D., i F. S. STEPANENKO. Iarovizatsiia sel'skokhoziaistvennykh rastenii. 64 pp. Gosudarstvennoe izdatel'stvo kolkhoznoi i sovkhoznoi literatury, Moskva. 1935.
15. MCKINNEY, H. H., W. J. SANDO, ARTHUR F. SWANSON, VINCENT C. HUBBARD, GLENN S. SMITH, COIT A. SUNESON, and JOE L. SUTHERLAND. Field experiments with vernalized wheat. U. S. Dept. Agric. Circ. 325. 7 pp. 1934.
16. MARTIN, JOHN H. The practical application of iarovization. Jour. Amer. Soc. Agron. **26**: 251. 1934.
17. MAXIMOV, N. A., i M. A. KROIKINA. Issledovanii͡a nad posledeistviem ponizhennoi temperatury na dlinu vegetatsionnogo perioda. Bull. App. Bot. Genet. & Plant-breed. **23**(4): 427–473. 1929–1930.
18. MAXIMOV, N. A., i A. I. POJARKOVA. K voprosu o fiziologicheskoi prirode razlichii mezhdu iarovymi i ozimymi rasami khlebnykh zlakov. Bull. App. Bot. Genet. & Plant-breed. **14**(1): 211–234. 1924–1925.
19. MURINOV', A, Koloshenle ozimykh' rzhi [i] [p]shenitsy pri iarovom' posievie. Zhur. Opytnoi Agron. **14**: 238–254. 1913.
20. PELTIER, GEORGE L., and T. A. KIESSELBACH. Inducing early fruiting in winter wheat by seed activation. Jour. Amer. Soc. Agron. **26**: 503–506. 1934.
21. POJARKOVA, A. J. Temperaturbedingungen der Keimung als bestimmender Faktor für Ährenbildung beim Wintergetreide. Ber. Deutsch. Bot. Ges. **45**: 627–637. 1927.

22. PURVIS, OLIVE NORAH. An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. **48**: 919–956. 1934.

23. TOLMATSCHEV, M. Zur Frage der physiologischen Natur der Stengelbildung beim Winter Korn und bei der Zuckerrübe. Proc. U.S.S.R. Congr. Genet., Plant and Animal Breed. Leningrad, 1929. **3**: 539–553. 1929.

24. UKRAINSKII INSTITUT SELEKTSII. Instruktsiîâ po iarovizatsii tverdykh îârovykh pshenits. Biull. Iarovizatsii **1**: 62–70.

25. WACAR, B. A. Zur Frage über d. Einfluss Temperatur auf die Bildung der Aehren bei Winterroggen und Winterweizen. Jour. Landw. Wiss. **2**: 776–785. 1925.

COMPOSTS

M. M. McCool

There is a great need for the utilization of plant materials to ameliorate the physical condition and to stimulate the microbial activities of soils and to increase their content of plant nutrients. There are also the probabilities that growth-promoting substances are present in plant materials, or are formed during their decomposition.

Some plant materials, owing to their coarse texture, are not suitable for use until composted. If a crop is planted soon after liberal applications of such plant materials are made to the soil, its development may be retarded greatly. As a result much valuable organic matter is burned or otherwise wasted and does not reach the soil.

The injury to growing plants caused by incorporating crude organic matter into the soil is considered to be mainly the result of competition for nitrogen between the micro-organisms of decomposition and the growing plants (15, p. 516; 17, 1). These injurious effects may be counteracted and the rate of decomposition increased by the addition of nitrogen in readily available form.

LITERATURE REVIEW

It was recognized many years ago in this country that it was possible to improve plant materials by composting them with nitrogenous fertilizers. According to Dachnowski (3), Samuel Johnson in 1866 called attention to satisfactory results which were obtained by composting muck with fish and applying the resulting compost to the soil. J. C. Neal (8) in 1889 discussed the composting of muck with manure and cotton seed, and set forth 11 formulae for preparing the compost.

The researches of Hutchinson and Richards (6) at the Rothamsted Agricultural Experiment Station, England, did much to arouse interest in the preparation of composts. These authors emphasized the importance of air supply, suitable temperature, a suitable supply of soluble nitrogen, and a neutral or alkaline reaction. Several carriers of nitrogen were used successfully by them, notably calcium cyanamid, urea, ammonium carbonate, peptone, and ammonium sulphate. According to these investigators, if either insufficient or too much available nitrogen is added, decay proceeds slowly. If too much nitrogen is used its loss by volatilization results, after which the rate of decomposition increases.

Richards and Norman (10, p. 1769) determined experimentally the nitrogen factor for a number of materials. The nitrogen factor was considered to be "the maximum amount of nitrogen, which when added to decomposing material, will be retained without loss." The factors ranged

from 0.12 to 1.33 pounds of nitrogen per 100 pounds of material. They held that the nitrogen factor cannot be predicted, but must be determined experimentally. Viswanatha Ayyar (14) dissolved 50 pounds of calcium cyanamid in water and added it to 1500 pounds of paddy straw. Sixty-nine days later the compost was brownish-black in color, and resembled well rotted farmyard manure. Smith, Stevenson, and Brown (12) reported results of investigations over a five-year period on the production of artificial manure from oat straw and corn stalks. They added six-tenths per cent nitrogen as ammonium sulphate, calcium cyanamid, and "Adco" reagent (a trade mixture, the nitrogen of which is calcium cyanamid). The cyanamid-treated straw decomposed at a slightly slower rate than did that to which the other carriers of nitrogen were added. The cyanamid compost, however, contained more nitrogen than did the others. It was reported by the Iowa Agricultural Experiment Station workers in 1929 (9, p. 15), "Adco and mixtures of cyanamid were the most effective reagents tried for stimulating decomposition and producing the best manure." The same organization reported in 1930 (5, p. 85), "Further work with artificial manures has shown that a good grade of manure can be made by composting straw with a mixture of cyanamid and rock phosphate in equal proportions without the addition of water, except as furnished by the rainfall, provided a normal rainfall is secured and the compost is allowed to decompose through the second year." Waksman and Tenney (16) studied the rate of decay of oak leaves and pine needles (*Pinus strobus*), over a short period, by placing 5 grams in flasks, and incubating them at temperatures ranging from 25° to 27° C. The addition of mixtures of ammonium and potassium phosphates increased their rate of decomposition. These materials, however, decayed much more slowly than did rye straw.

Tenney and Waksman (13) found upon the addition of 1 gram of $NH_4H_2PO_4$, 1 gram K_2HPO_4, and 2 grams of $CaCO_3$ to 200 to 280 grams of dry, mature oak leaves, no appreciable increase in the rate of decomposition over that which took place in the untreated leaves.

McLean (7) treated salt marsh hay with "Adco" reagent at the rate of 150 pounds per ton. The salts were added to one-foot layers of moistened hay and heaps 12×30×6 feet were made. Difficulty in keeping the heaps uniformly moist and obtaining uniform compost was encountered. Fresh hay also was employed. That treated with "Adco" reagent had settled to about 1/6 of its original volume after three months, was dark brown in color, and contained 1.89 per cent nitrogen.

Richards and Shirkhande (11) in their studies on the utilization of different forms of inorganic nitrogen in the decomposition of plant materials found a preference, by the organisms concerned in the early stages of decomposition of straw, for nitrogen in the form of ammonia rather

than in the form of nitrate. During later stages, this preference was not manifested. The nitrogen factor was lower for nitrate than it was for ammonia nitrogen, and the loss of nitrogen was greatest when nitrate was present.

More recently Demolon and Burgevin (4) made up three formulae, one including calcium cyanamid, and gave plans for the construction of a container for the composts.

MATERIALS AND METHODS

The organic materials used in this study were as follows: needles of pine trees (*Pinus taeda* L.); leaves of white oak (*Quercus alba* L.); leaves of Norway maple (*Acer plantoides* L.); leaves of sugar maple (*Acer saccharum* L.); hay from salt marsh grass or black grass (*Juncus gerardi* Loisel); cat-tail (*Typha latifolia* L.); straw of wheat (*Triticum aestivum* L.); and peat. The effects of these materials, when used as such and in composted form, were determined by growing rye (*Secale cereale* L.), rye grass (*Lolium perenne* L.), privet (*Ligustrum ovalifolium* Hassk.) cuttings, tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L. var. Turkish). Determinations were also made of the pH values and the nitrate contents of the soils treated with these materials and the composts produced from them.

The pine needles were obtained through the courtesy of County Agent W. O. Strong, Onley, Virginia. They were collected in November 1934. The salt marsh hay was harvested from a salt marsh near the eastern shore of New Jersey and shipped to the Boyce Thompson Institute by J. C. Schoemaker of Bridgeton, New Jersey. The leaves were collected locally during the first week of December. The cat-tails were harvested just prior to the appearance of the flowering stems. The peat was furnished by the Hyper Humus Company, Newton, New Jersey.

Boxes, each dimension of which was three feet, were used as containers for leaves, wheat straw, and salt marsh hay. Unless stated otherwise, the peat, pine needles, and cat-tails were composted in 12-gallon glazed jars. The cat-tails were chopped into pieces about one inch long, and placed in the containers in the fresh condition. The materials, except the peat, were sprinkled with a fine spray until they would not take up additional water. Twenty-four hours after the addition of the water, the fertilizer salts were mixed throughout, unless otherwise stated. All except the peat were packed rather firmly in their containers. Waxed paper was spread on the surface of the contents of the boxes, and earthenware covers were placed on the jars. This procedure greatly reduced the loss of water by evaporation, especially during the early stages of decomposition of marsh hay, leaves, and wheat straw.

The methods employed by Richards and Shirkande (11) were followed

in determining the contents of nitrate and ammonia nitrogen in the composts. The method followed by J. W. Ames of the Ohio Agricultural Experiment Station was used in determining total nitrogen in fresh manure. About 5000 grams of the well mixed compost were finely chopped,

TABLE I

TREATMENT OF COMPOSTS

Compost No.	Material	Amounts per 2000 lbs. dry matter	
		Cyanamid, lbs.	Superphosphate, lbs.
1	Wheat straw	0	0
2	" "	140	100
3	" "	140	200
4	" "	70	100
5	" "	70	50
6	" "	50	50
7	Mixed leaves	0	0
8	" "	70	50
9	" "	70	50*
10	White & red oak leaves	0	0
11	" " " " "	56 5	0
12	" " " " "	113	0
13	" " " " "	56 5	28 2
14	Sugar maple leaves	0	0
15	" " "	75	37 5
16	Norway maple leaves	0	0
17	" " "	100	40
18	Salt marsh hay	0	0
19	" " "	70	50
20	" " "	70	100
21	" " "	70	100**
22	" " "	70	0
23	" " "	50	50
24	" " "	40	80
25	Pine needles	0	0
26	" "	140	140
27	" "	140	70
28	" "	70	140
29	" "	70	70
30	" "	70	70**
31	Peat	0	0
32	"	200	200
33	"	200	100
34	"	100	200
35	"	100	50
36	"	70	100***
37	"	140	100***

* Fertilizer salts placed in layers 4, 12, and 24 inches from the surface.
** One part of fresh horse manure mixed with 50 parts of compost.
*** Heaps 5 cubic yards each.

mixed, and 500 grams put through a food grinder, mixed, and 10 grams transferred to a Kjeldahl flask, treated with 25 cc. sulphuric acid, let stand overnight, and the nitrogen determined as usual. The quinhydrone method was followed in determining the pH values.

Leaves and pine needles were mixed with 12 pounds of soil to determine their effect on the nitrate content of Gloucester loam. The mixture was placed in 2-gallon glazed jars, at temperatures ranging from 72° to 78° Fahrenheit. The moisture content was maintained at 18 per cent. The nitrates in one-to-five water extracts were determined colorimetrically.

The fertilizing value of the composts was determined by means of soil cultures. Unless otherwise stated, 2-gallon glazed jars were filled with Gloucester loam with which the fertilizer salts and composts had been thoroughly mixed. Twenty carefully selected Rosen-rye seed were planted, and after the seedlings were well established the number in each container was reduced to eight. Where the effects of leaf compost on the development of privet were determined, the compost was mixed with material taken from the B horizon of Gloucester loam. Uniform cuttings, each of which had a slight root development, were set in the containers.

The dry-weight percentages of organic matter and nitrogen in the various materials were as follows: pine needles, organic matter 97.60, nitrogen, 0.588; salt marsh hay 93.87, 0.497; oak leaves 94.07, 0.868; Norway maple leaves 88.23, 0.812; sugar maple leaves 92.61, 0.74; wheat straw 91.04, 0.54; cat-tail 90.96, 2.023; peat 90.32, 3.91. The compost treatments are given in Table I.

EXPERIMENTAL RESULTS

Changes in the pH values of mixtures of soil and plant materials. The changes in the pH values of soils and mixtures consisting of one part by volume of plant material and three parts of soil were ascertained. According to the data in Table II, the mixtures, one day after the experiments

TABLE II
EFFECT OF PLANT MATERIALS ON THE pH VALUES OF SOILS

Material added to soil	Podunk loam				Norfolk fine sand			
	1 day	15 days	30 days	45 days	1 day	15 days	30 days	45 days
None	4.95	5.07	5.19	4.95	5.93	6.02	6.56	6.47
Norway maple leaves	4.53	4.99	5.63	5.88	4.87	5.54	6.84	6.98
Sugar maple leaves	4.27	4.78	5.29	5.29	4.29	4.82	6.14	6.31
Pine needles	4.70	4.95	5.29	5.12	4.65	5.37	6.14	5.97
Oak leaves	4.44	4.60	4.95	5.46	5.04	5.54	6.39	6.74

were begun, were more acid, as judged by the pH values, than was the Podunk soil. After fifteen days, the soil plus Norway maple leaves and soil plus pine needles had about the same reaction as the untreated soil, but at this time those made up of soil plus sugar maple leaves and soil plus oak leaves were more acid. Fifteen days later the pH values of the Norway maple were higher than those of the untreated soil. At the close of the ex-

periment, the notable change that had taken place was in the soil and oak leaf mixture, which had become less acid than the soil alone. The changes in the pH values of the Norfolk fine sand series of cultures proceeded in the same direction as did those of the Podunk loam series. The soil and Norway maple leaf mixtures were higher in pH after 30 days than were the untreated soils. At this time, the untreated soil cultures were less acid than were the other mixtures. At the termination of the experiment, the untreated soil was notably less acid than the mixture made up of sand plus pine needles.

Effect of plant materials on the nitrate content of soil. The effect of ground Norway maple leaves, sugar maple leaves, pine needles, and a mixture of red oak and white oak leaves on the nitrate content of Gloucester loam was ascertained. The water extract of the soil contained 81.6 parts per million of nitrates after 15 days, and 136.1 parts per million after 30 days. There were only slight traces of nitrates in the extracts from 2-gallon jars of soil with which had been mixed 20, 40, and 80 grams of oak leaves; 80 grams of oak leaves which had been composted 150 days; 40 and 80 grams of Norway maple leaves; 40 and 80 grams of sugar maple leaves; and 80 grams of pine needles, respectively.

Temperature changes. The changes in temperature 12 inches from the surface of wheat straw and marsh hay composts were ascertained after various intervals of time. Table III gives the temperature records of the former. It should be noted that the straw composts were removed from their containers, moistened, mixed, and returned 22 and 34 days after they were laid down. The temperature of the untreated straw rose and fell

TABLE III

TEMPERATURES IN STRAW COMPOSTS, ° F.

Room temp., ° F.	Incubation period, days	Compost number*					
		1	2	3	4	5	6
63	6	154	82	68	118	97	104
62	7	142	102	70	130	112	114
62	10	130	128	78	142	132	134
63	12	120	140	98	144	124	136
62	15	122	134	105	126	128	138
62	18	95	124	120	118	124	126
64	23	88	144	146	138	120	140
63	30	98	110	108	102	103	104
63	37	100	102	116	96	98	110
65	49	78	68	65	66	65	64
66	54	76	69	70	67	68	66

* 1 = untreated straw
2 = straw + 140 lbs. of cyanamid + 100 lbs. phosphate per ton
3 = straw + 140 lbs. cyanamid + 200 lbs. phosphate
4 = straw + 70 lbs. cyanamid + 100 lbs. phosphate
5 = straw + 70 lbs. cyanamid + 50 lbs. phosphate
6 = straw + 50 lbs. cyanamid + 50 lbs. phosphate, placed in layers 4, 12, and 24 inches from the surface.

rapidly. That of compost 3, the one which received 140 pounds of calcium cyanamid and 200 pounds of superphosphate per ton, rose much more slowly than did that of the other treated ones. The rate at which heat was given off by each of the treated composts decreased rapidly about 30 days after they were placed in the containers. After about 50 days, they attained the temperature of the room in which they were incubated, and so remained until the close of the experiment.

Temperature changes were recorded in untreated salt marsh hay and hay with which 40 pounds of calcium cyanamid and 80 pounds of superphosphate per ton were mixed. The temperature of the untreated hay rose and fell more rapidly at the beginning of the experiment and after stirring than did that of the fertilized hay.

The temperature changes of the leaf composts were not so great as were those of the wheat straw and salt marsh hay composts. They, however, gave off heat over a longer period. Those treated with cyanamid were more active in this respect than were the untreated leaves. The maximum temperature of the Norway maple leaf composts was lower than that of the other materials. Mixing the composts did not result in such striking changes in temperature as resulted when the straw and hay composts were mixed.

Physical changes. The fertilized straw composts soon became light gray in color to a depth of 20 inches, due to the presence of fungi. As the fungi disappeared, the temperature declined, the color changed gradually to light brown, the bulk decreased, and structural changes took place. After about 100 days, the differences in color, structure, and volume of the differently treated composts were insignificant. The final products were very dark brown in color, and were highly colloidal.

The untreated straw compost, after about 30 days, was dark brown in color to a depth of six inches. The remainder was light brown. The volume had decreased less than 10 per cent, and the structure had not visibly changed. Seventy days later, the color was brown and the volume had decreased about 25 per cent.

The color of the treated salt marsh hay in the containers first became gray, then light brown, and finally dark brown. Although the visible structural changes occurred later than they did in the straw composts, once they began to take place they proceeded rapidly. The final products were similar and highly colloidal. The transformations which took place in the untreated hay were much less marked.

It was found difficult, in cooperative experiments with J. C. Schoemaker, to prevent the loss of moisture from large heaps of salt marsh hay, and as a result the rate of decay was unsatisfactory. In order to accomplish this, the heaps either should be covered, or the fresh material should be employed. Another procedure would be to add nitrogen to the hay in the pound after having served as bedding for livestock.

The untreated oak leaf composts decreased about 10 per cent in volume. Their structure was altered only slightly during the period of observation. Those treated with cyanamid, and cyanamid plus superphosphate, decreased about 33 per cent in volume, were dark brown in color, and were easily broken up upon mixing and screening. The mixtures which were not disturbed during the period of incubation decreased in volume slightly less than did those which were removed and mixed.

The untreated sugar maple leaves broke down somewhat, and became darker in color upon standing. Those which received cyanamid and super-

FIGURE 1. Upper left to right: untreated composts of sugar maple leaves, Norway maple leaves, oak leaves. Lower left to right: sugar maple leaves + 75 lbs. of calcium cyanamid + 40.5 lbs. of superphosphate; Norway maple leaves + 100 lbs of calcium cyanamid + 37.5 lbs. of superphosphate; oak leaves + 56.5 lbs. of calcium cyanamid + 28.2 lbs. superphosphate per ton respectively.

phosphate had largely disintegrated after about 50 days. The volume decrease of the former was about 10 and of the latter 40 per cent.

The untreated Norway maple leaves had decayed only slightly. The decrease in volume was approximately 5 per cent after 100 days. At this time, the treated compost broke up readily when forked or screened. The color was dark brown, and the volume about 65 per cent of the original. Changes in the structure of the leaf composts are illustrated in Figure 1.

The decrease in bulk and the physical changes of pine needle composts in the containers took place very slowly, and were not great. The treated needles became brown and finally dark brown in color. The color changes, together with the ease with which they broke down when handled, were the outstanding visible alterations. The color of the untreated needles changed only slightly, decreased less in bulk, and their resistance to breaking decreased to a less extent than did the treated ones.

Tests with pine needles also were conducted in cooperation with County Agent W. O. Strong at Onley, Virginia. The needles were moistened, and 75 pounds of cyanamid, 100 pounds of superphosphate, a small amount of fresh horse manure, and decayed vegetable matter from the forest were mixed with 650 cubic feet of needles. Owing to their porous nature, the needles lost water rapidly, and the results obtained were not satisfactory. In another trial, the needles were used as bedding in the stable, and thrown into the pound. As each foot of residue accumulated, cyanamid and superphosphate were scattered over one-half the surface of the pound. The structure of the treated portion was altered to a greater extent than was that of the materials which did not receive the fertilizer salts.

The volume of the cat-tail composts treated with cyanamid, superphosphate, and potassium chloride decreased more rapidly than did the composts which did not receive the calcium cyanamid. They soon became light brown, as the period of incubation increased, dark brown, and finally brownish-black in color. At the close of the experiment they were slightly fibrous, highly colloidal, and intractable when dry. At this time, the compost to which cyanamid was not applied was light brown in color, and mainly fibrous.

Changes in pH values of composts. The pH values of fresh or untreated and composted mixtures of white oak and red oak leaves, sugar maple leaves, Norway maple leaves, and salt marsh hay were determined. The pH values of the untreated leaves were all low before composting, but became greater upon standing. After 151 days, they were 7.46, 6.78, and 7.42 in the order given. The composts which contained the fertilizer salts soon became less acid than the controls, but at the final period they were alkaline in reaction and did not vary greatly.

The pH value of the untreated salt marsh hay was 7.83, and rose slightly upon standing. The values for the fertilized hay were slightly less than those of the control throughout the experiment.

Nitrogen changes. The changes in the percentage of total nitrate and ammonia nitrogen in some of the composts were ascertained after different intervals of time had elapsed. Some of the results obtained are given in Table IV. The percentage of nitrogen in each increased as the experiment progressed. The amount of nitrogen in the form of nitrate and ammonia

TABLE IV

CHANGES IN NITROGEN AND ASH CONTENTS OF COMPOSTS

Material and treatment per ton	Incubation period, days	% of dry matter			
		Nitrogen	NO_3	NH_3	Ash
Wheat straw. No treatment	34	1.239	0.083	0.140	14.92
	73	1.303	0.065	0.009	16.70
	104	1.358		0.014	16.64
Wheat straw+140 lbs. calcium cyanamid+100 lbs. superphosphate	34	2.512	0.794	0.197	30.70
	73	3.120	0.614	0.207	38.21
	104	3.253	0.045	0.231	40.43
Salt marsh hay. No treatment	50	1.316	0.058	0.051	12.94
	82	1.414	0.078	0.064	12.36
	105	1.631		0.064	16.35
Salt marsh hay+70 lbs. calcium cyanamid+100 lbs. superphosphate	69	2.431	0.142	0.107	20.1
	83	2.562	0.101	0.126	19.6
	368	3.274	1.294	0.037	30.3
Pine needles. No treatment	368	1.057	0.091	0.011	10.7
Pine needles+70 lbs. calcium cyanamid+70 lbs. superphosphate	276	2.540	1.562	0.103	17.81
	368	3.129	1.860	0.049	20.34
White oak and red oak leaves. No treatment	50	0.952	0.074	0.003	14.96
	82	1.008	0.071	0.002	12.04
	105	1.008	0.051	0.002	14.67
	152	2.135	0.078	0.048	16.21
White oak and red oak leaves+113 lb. calcium cyanamid	50	2.471	0.102	0.086	16.97
	82	2.744	0.065	0.076	19.01
	105	2.798	0.352	0.061	17.38
	152	2.926	0.597	0.057	21.80
Sugar maple leaves. No treatment	50	1.344	0.139	0.071	19.54
	82	1.596	0.080		15.65
	105	1.596		0.009	17.65
	152	1.708	0.095	0.016	19.05
Sugar maple leaves+75 lbs. calcium cyanamid+37.5 lbs. superphosphate	50	2.590	0.079	0.003	14.28
	82	2.870	0.089	0.079	22.02
	105	3.122		0.087	20.98
	152	3.094	0.504	0.061	23.36
Norway maple leaves. No treatment	50	0.924	0.063	0.003	18.09
	82	1.064	0.063	0.003	20.14
	105	1.190		0.005	17.44
	152	2.940	0.091	0.025	22.98
Norway maple leaves+100 lbs. calcium cyanamid+40 lbs. superphosphate	50	2.079	0.089	0.079	24.23
	82	2.401	0.081	0.055	25.09
	105	2.408		0.034	26.07
	152	2.562	1.351	0.037	29.24

in the untreated wheat straw, pine needles, and salt marsh hay were small, and did not vary markedly. The percentage of nitrates in the treated wheat straw decreased markedly, and the percentage of ammonia increased somewhat upon standing, whereas the reverse was true for the heavily fertilized salt marsh hay and pine needles. The changes in the amount of these in the salt marsh hay treated with a small amount of calcium cyanamid were not great.

The changes in the nitrate content of the untreated oak, Norway maple, and sugar maple leaves were slight. The amounts of nitrates in composts of oak leaves, to which cyanamid was added at the rate of 56.5 pounds per ton, and in composts with which this amount of cyanamid and superphosphate was mixed at the rate of 28.2 pounds per ton, remained about the same during the composting period. The addition of double this amount of cyanamid, however, resulted in marked increases in the content of this form of nitrogen. The nitrate content of composts of Norway maple leaves and sugar maple leaves, to which cyanamid and superphosphate had been added, increased with the extension of the incubation period. The percentage of ammonia nitrogen in the leaf composts was comparatively low throughout the experiment. The nitrogen content of each of the leaf composts was highest at the final period of sampling, or 151 days after they were placed in the containers. Samples of fertilized salt marsh hay, oak leaves, and sugar maple leaves lost 13.3, 11.9, and 13.9 per cent of their total nitrogen when dried overnight in an oven, the temperature of which was 103° C. Air drying resulted in somewhat smaller losses of this element.

The changes in the percentages of the foregoing forms of nitrogen in three cat-tail composts were determined. The compost with which superphosphate and potassium chloride were mixed at the rates of 100 and 50 pounds, respectively, per ton of dry material, showed increases in the percentage of total and nitrate nitrogen, and a rise followed by a decline in the ammonia nitrogen. The inclusion of cyanamid in this mixture retarded the accumulation of nitrates, but the percentage of ammonia nitrogen was increased by it as the period of incubation was extended, and increased the percentage of nitrogen from 3.84 to 4.92.

It is apparent from these studies that these plant materials may be composted with widely varying amounts of cyanamid. Indications are that addition of superphosphate along with the nitrogen carrier does not materially speed up the rate of decomposition. Its presence, however, may reduce the loss of nitrogen and improve the composts for some purposes. If compost heaps are constructed about five feet wide and any convenient height, and if the fertilizer salts are mixed throughout the heap, one mixing about 30 days after the composts are laid down should suffice.

PLANT GROWTH STUDIES

The effect of different composts on the growth of rye, rye grass, and rooted privet cuttings was determined by means of soil cultures.

Comparison of composts. The data derived from the first set of cultures in which fertilized pine needle, salt marsh hay, and mixed leaf composts were employed are summarized in Table V. The outstanding facts are

TABLE V

EFFECT OF COMPOSTS ON THE GROWTH OF RYE; DURATION OF GROWTH PERIOD 104 DAYS

Cultural treatment	Dry wt. in grams			
	Replicates			Total
Control, P.K.	12.5	11.3	12.1	35.9
40 g. in pine needles, P.K. not composted	9.0	8.5	9.2	26.7
0.8 g. nitrogen in pine needle compost (70 lbs. cyanamid+140 lbs. superphosphate per ton)+K.	20.0	19.2	18.9	58.1
0.8 g. nitrogen in pine needle compost (70 lbs. cyanamid+70 lbs. superphosphate per ton)+K.	18.1	17.8	16.2	52.1
40 g. marsh hay, P.K.	5.5	5.8	4.2	15.5
0.8 g. nitrogen in marsh hay compost (70 lbs. cyanamid+50 lbs. superphosphate per ton)+K.	17.2	16.8	17.4	51.4
1.6 g. nitrogen in marsh hay compost (70 lbs. cyanamid+50 lbs. superphosphate per ton)+K.	22.7	18.5	20.2	61.4
0.8 g. nitrogen in mixed leaf compost (70 lbs. cyanamid+50 lbs. superphosphate per ton)+K.	17.6	16.8	15.8	50.2
0.8 g. nitrogen in straw compost (70 lbs. cyanamid +100 lbs. superphosphate per ton)+K.	16.7	16.5	18.3	51.5
0.8 g. nitrogen in shredded manure+K.	14.1	15.2	14.8	44.1

the similarity of results obtained from the composts and their superior value in comparison with shredded stock-yard manure. It should be noted also that the addition of undecomposed salt marsh hay and pine needles to the soil decreased markedly the yields of rye.

The effect of time of composting wheat straw on the yields of rye was determined by means of another set of soil cultures. According to the results presented in Table VI, it was not necessary to incubate the materials until the straw had gone far in its decomposition, inasmuch as the differences in the yields derived from the addition of an equivalent amount of nitrogen in the mixture which had been composted 30, 46, and 103 days, respectively, were insignificant. It was found advisable, however, to break up the coarse material before adding it to the soil. Further, the New York

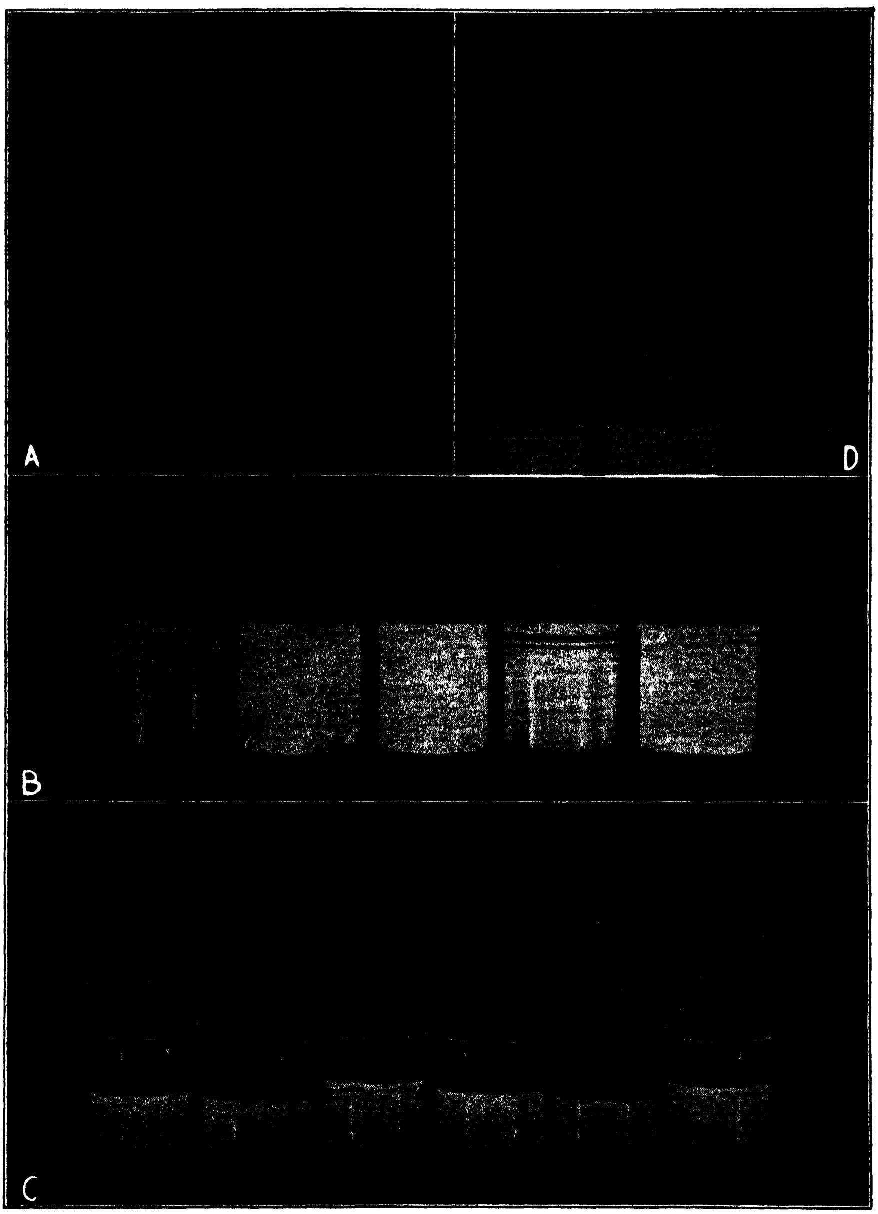


Figure 2. Effects of composts on growth. A, privet cuttings; B, rye grass; C and D, rye. (For details of treatment of the various lots see text.)

TABLE VI

EFFECT OF DURATION OF INCUBATION PERIOD ON THE FERTILIZING VALUE OF WHEAT STRAW COMPOST; GROWTH INDICATOR RYE

Cultural treatment*	Length of incubation period, days	Dry wt. of three cultures, g.
Soil		36.2
Soil+0.5 g. of nitrogen in compost	30	58.2
Soil+0.5 g. of nitrogen in compost	46	61.0
Soil+0.5 g. of nitrogen in compost	103	58.0
Soil+0.8 g. of nitrogen in compost	103	86.4

* Compost made up of 70 lbs. of cyanamid+50 lbs. of superphosphate per ton of dry straw. All cultures received 10 g. of superphosphate +0.5 g. of potassium sulphate.

State College of Agriculture (2) notes that when artificial manure and animal manure are used under field conditions, fall applications are advisable.

Effect of leaf compost on the growth of privet. The effect of composts on the root development of privet cuttings is shown in Figure 2 A. In Figure 2 A reading left to right the treatment given was as follows: control; soil+200 g. oak leaves composted 70 days with 56.5 lbs. calcium cyanamid +28.2 lbs. superphosphate; and soil+400 g. of the same material. Period of growth 80 days. Basic treatment of all cultures 10 g. superphosphate +0.5 g. KCl.

TABLE VII

EFFECT OF TOP-DRESSING RYE GRASS WITH NEEDLE AND LEAF COMPOSTS

Cultural treatment*	Yields in dry wt., g.			Total, g.
	1st cutting	2nd cutting	3rd cutting	
Soil	0.36	0.30	0.240	0.90
10 lbs. moist needle compost per 100 sq. ft.**	1.545	0.840	0.390	2.77
20 lbs. moist needle compost per 100 sq. ft.**	2.07	0.93	0.54	3.54
40 lbs. moist needle compost per 100 sq. ft.**	3.42	1.335	0.57	5.32
10 lbs. moist leaf compost per 100 sq. ft.***	0.885	0.610	0.39	1.84
20 lbs. moist leaf compost per 100 sq ft.***	1.020	0.690	0.54	2.25
40 lbs. moist leaf compost per 100 sq. ft.***	2.250	0.750	0.57	3.57

* All cultures received 10 g. of superphosphate+0.5 g. of potassium sulphate.

** Compost mixture 70 lbs. calcium cyanamid+140 lbs. superphosphate per ton dry needles; composted 300 days.

*** Compost mixture 70 lbs. calcium cyanamid+50 lbs. superphosphate per ton dry leaves; composted 300 days.

Effect of composts on the growth of rye grass. Rye grass was grown several weeks in jars of Gloucester loam after which it was clipped and then top-dressed with different amounts of screened pine needle and mixed leaf composts. The dry weights of the plants derived from the cultures are given in Table VII. The composts increased the yields of each of three cuttings of grass, but the effects on the yields of the first harvest were most striking.

Owing to their physical nature, these materials reduced greatly the amount of water evaporated from the surface of the soil. Oak, sugar maple, and Norway maple leaves composted 100 days with fertilizer salts, proved to be excellent materials for top-dressing rye grass turf. The growth effects of composts on rye grass are shown in Figure 2 B, in which the treatments were as follows (left to right): control; oak leaves composted with 56.5 lbs. of calcium cyanamid+28.2 lbs. superphosphate; pulverized stockyard manure; sugar maple leaves composted with 75 lbs. calcium cyanamid +37.5 lbs. of superphosphate; Norway maple leaves composted 75 days with 100 lbs. calcium cyanamid+40 lbs. superphosphate per ton. The composts were applied at the rate of 32 g. of dry matter per culture.

TABLE VIII

EFFECT OF FRESH AND COMPOSTED LEAVES ON THE EARLY GROWTH OF RYE AFTER 64 DAYS

Material	Amounts added per culture, g.	Yields in dry wt., g. Replicates			Total, g.
0	0	6.6	5.8	6.4	18.8
Oak leaves	20	5.6	4.9	5.3	15.8
	40	3.2	2.6	2.8	8.6
Oak leaves composted 105 days	40	3.1	3.2	4.2	10.5
	80	1.7	1.4	1.2	4.3
Sugar maple leaves	40	1.8	1.9	1.4	5.1
	80	0.6	1.1	0.8	2.5
Sugar maple leaves composted 105 days	40	4.6	4.2	4.8	13.6
	80	3.2	3.1	3.4	9.7
Norway maple leaves	40	2.2	2.6	2.4	7.2
Norway maple leaves composted 105 days	40	5.1	6.2	4.5	15.8
	80	3.2	3.1	3.5	9.8

Although the results are not described fully here, the leaf compost proved to be excellent for the production of several potted flowering plants, when one part was mixed with three parts of Gloucester loam, to 500 parts of which had been added 1 part of a 4–8–7 commercial fertilizer.

The fertilizing value of leaves and composted leaves, as measured by the early growth of rye, was determined by means of soil cultures. An examination of the data in Table VIII reveals that 20 grams of a mixture of air-dried, fresh or uncomposted red oak and white oak leaves, when mixed with 5,600 grams of Gloucester loam, decreased production slightly,

and 40 grams greatly retarded the growth of rye plants. Composting these 105 days without the addition of fertilizer salts did not alter the growth effects markedly. Sugar maple and Norway maple leaves retarded the development of rye plants to a greater extent than did the oak leaves. Their inhibitive action, however, was reduced to a greater degree by composting.

The effect of composts, obtained by composting for 105 days, on the growth of rye is shown in Figure 2 C. The treatments shown in this figure, together with the yields of triplicate cultures, were as follows (left to right): soil, no treatment, 10.2 g.; soil+1.6 g. nitrogen in red and white oak leaves composted with 56.5 lbs. of calcium cyanamid+28.2 g. superphosphate, 25.2 g.; soil+1.6 g. of nitrogen in sugar maple leaves composted with 75 lbs. calcium cyanamid+37.5 lbs. of superphosphate, 29.7 g.; soil+1.6 g. of nitrogen in Norway maple leaves composted with 100 lbs. calcium cyanamid+40 lbs. superphosphate, 30.6 g.; soil+1.6 g. nitrogen in salt marsh hay composted with 40 lbs. calcium cyanamid+80 lbs. superphosphate, 40.2 g.; soil+1.6 g. nitrogen in white and red oak leaves composted with 113 lbs. calcium cyanamid per ton dry material, 39.7 g. The yield from the lot receiving soil+1.6 g. of nitrogen in pulverized manure was 32.2 g., showing that composting these materials with fertilizer salts 105 days resulted in materials which compared favorably in fertilizing value with pulverized stock-yard manure. It should be noted that all cultures received 15 grams of superphosphate and 0.5 gram of potassium sulphate.

TABLE IX

EFFECT OF PEAT COMPOSTS ON THE GROWTH OF RYE AFTER 100 DAYS

Cultural treatment*	Yields in dry wt., g.			Total, g.
	Replicates			
Soil**	9.6	10.2	10.4	30.2
0.8 g. nitrogen in compost 32	14.5	15.4	14.6	44.5
1.6 g. nitrogen in compost 32	18.0	18.2	19.3	55.5
0.8 g. nitrogen in compost 33	8.4	8.3	8.7	25.4
0.8 g. nitrogen in compost 34	12.8	13.5	13.0	39.0
0.8 g. nitrogen in compost 35	4.0	4.5	5.2	13.7

* Compost 32 was made up of peat+200 lbs. calcium cyanamid+200 lbs. of superphosphate per ton of dry matter; compost 33 consisted of peat+200 lbs. of calcium cyanamid+100 lbs. of superphosphate; compost 34, peat+100 lbs. calcium cyanamid+200 lbs. superphosphate; and compost 35, peat+100 lbs. calcium cyanamid+50 lbs. superphosphate.

** 10 g. of superphosphate and 0.5 g. KCl per culture.

Growth tests with peat composts. Peat, the water content of which was 70 per cent, was composted 240 days in 12-gallon jars with cyanamid and superphosphate in different ratios, and the effect of the resulting composts on the growth of rye in soil culture was ascertained. According to the data

in Table IX, peat composted with mixtures in which superphosphate was equal to or greater than cyanamid, was favorable for the growth of rye. The materials which resulted from composting the peat with mixtures containing less superphosphate than cyanamid retarded the development of this crop. Similar effects on wheat, tomato, and tobacco plants were observed. Peats, the water contents of which were 58 and 80 per cent, respectively, were also composted in 12-gallon jars with equal parts of cyanamid and superphosphate. The resulting products were found to be toxic to rye and tomato plants.

Two peat composts, designated as A and B, five cubic yards each, were laid down Nov. 22, 1934, in a well-ventilated building at the Hyper Humus plant, Newton, New Jersey. Compost A received 35 pounds of granular cyanamid and 50 pounds of superphosphate,and compost B received 70 pounds of cyanamid and 50 pounds of superphosphate. Samples were taken 300 days later, and their effects on the growth of rye determined by means of soil cultures. It should be stated that their addition to Gloucester sandy loam soil resulted in greatly increasing the growth of this crop. The results obtained are illustrated by Figure 2 D, in which the treatments were as follows, (left to right): soil, no treatment; soil + 200 g. of peat compost A; soil + 200 g. of peat compost B.

SUMMARY

The pH values of soils to which composts of oak leaves, Norway maple leaves, sugar maple leaves, and pine needles, respectively, were added, were lower shortly after mixing than were those of the soils alone. They became less acid, however, upon standing. After 45 days the pH values of the soils containing composts of Norway maple leaves and oak leaves were higher than those of the soils alone.

Water extracts of Podunk loam which had been mixed with oak leaves, Norway maple leaves, sugar maple leaves, and pine needles, respectively, contained only slight traces of nitrates after 15 and 30 days.

The temperature of untreated straw and hay rose more rapidly upon moistening and mixing than did those to which fertilizer salts had been added.

Although the maximum temperatures of the leaf composts were lower than were those of the wheat straw and salt marsh hay composts, they gave off heat over longer periods of time. The presence of cyanamid increased their activity in this respect. The unfertilized oak leaf composts decomposed only slightly, followed in order by Norway maple leaf and sugar maple leaf composts. The addition of cyanamid to these materials resulted in striking changes in volume, color, and the ease of screening. The darkening in color and softening of the fertilized pine needle composts were notable changes. The cyanamid-treated cat-tail broke down rapidly,

the final products being dark brown in color, highly colloidal, and intractable when dry.

The pH values of the various leaf composts increased greatly upon standing. The salt marsh hay composts were alkaline in reaction throughout the experiment.

The percentage of nitrogen in all composts increased with time. With the exception of cat-tail, slight changes in the nitrate-nitrogen and ammonia-nitrogen contents of the untreated composts resulted. These forms of nitrogen in the treated composts varied with the treatments afforded and the kinds of plant materials.

It appears that the fertilizer salts should be mixed throughout the moist material which is to be composted, and the resulting mixture placed in heaps about five feet wide and four or more feet high.

The addition of uncomposted salt marsh hay, pine needles, oak leaves, sugar maple leaves, and Norway maple leaves to Gloucester loam retarded greatly the growth of rye. Composting 105 days decreased the injurious effects of oak leaves slightly, and those of Norway maple and sugar maple leaves to a somewhat greater extent.

Fertilized straw, hay, mixed leaf, oak leaf, sugar maple leaf, and Norway maple leaf composts increased greatly the yield of rye in Gloucester loam. Rooted privet cuttings responded strikingly in the development of roots and tops to applications of treated oak leaf composts. Fertilized pine needle, mixed leaf, oak leaf, sugar maple leaf, and Norway maple leaf composts were very effective when used as top-dressings on rye grass turf.

Domestic peat, with water contents 58 and 80 per cent, respectively, when composted with cyanamid, proved to be injurious to plants. When composted at a water content of 70 per cent with mixtures in which superphosphate was equal to or greater than cyanamid, it increased the growth of rye.

LITERATURE CITED

1. COLLISON, R. C., and H. J. CONN. The effect of straw on plant growth. New York [Geneva] Agric. Exp. Sta. Tech. Bull. 114. 35 pp. 1925.
2. Comparison of artificial manure and animal manure. *In* New York [Cornell] State Coll. of Agric. Ann. Rept. **1933**: 76. 1934.
3. DACHNOWSKI, ALFRED P. Preparation of peat composts. U. S. Dept. Agric. Dept. Circ. 252. 11 pp. 1922.
4. DEMOLON, A., et H. BURGEVIN. Instruction pratique pour la fabrication industrelle du fumier artificiel à partir de la paille de céréales. Syndicat professionel de l'industrie des engrais azotés. Bull. Périod. Renseignements Serv. Agric. Jan.–Feb. 1936: 3441–3444.
5. The formation of humus and the decomposition of organic matter in soils. *In* Iowa Agric. Exp. Sta. Ann. Rept. **1930–31**: 85. 1931.
6. HUTCHINSON, H. B., and E. H. RICHARDS. Artificial farmyard manure. Jour. Min. Agric. [Gt. Britain] **28**: 398–411. 1921.
7. MCLEAN, H. C. Making artificial manure from salt marsh hay by the "ADCO" process. New Jersey Agric. Exp. Sta. Rept. **1925–26**: 538–540. 1927.

8. NEAL, J. C. Compost-making. *In* Florida Agric. Exp. Sta. Bull. 7: 17–19. 1889.
9. The production of artificial manure. *In* Iowa Agric. Exp. Sta. Ann. Rept. **1928–29**: 14–15. 1929.
10. RICHARDS, ERIC HANNAFORD, and ARTHUR GEOFFREY NORMAN. The biological decomposition of plant materials. V. Some factors determining the quantity of nitrogen immobilised during decomposition. Biochem. Jour. **25**: 1769–1778. 1931.
11. RICHARDS, E. H., and J. G. SHIRKHANDE. The preferential utilization of different forms of inorganic nitrogen in the decomposition of plant materials. Soil Sci. **39**: 1–8. 1935.
12. SMITH, F. B., W. H. STEVENSON, and P. E. BROWN. The production of artificial farm manures. Iowa Agric. Exp. Sta. Res. Bull. 126. p. 165–195. 1930.
13. TENNEY, FLORENCE C., and SELMAN A. WAKSMAN. Decomposition of natural organic materials and their decomposition in the soil. IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials, under aerobic conditions. Soil Sci. **28**: 55–84. 1929.
14. VISWANATHA AYYAR, K. S. Note on the making of artificial farm-yard manure. Madras Dept. Agric. Yearbook **1923**: 21–25. 1924.
15. WAKSMAN, SELMAN A. Principles of soil microbiology. 897 pp. Williams & Wilkins Co., Baltimore, Md. 1927.
16. WAKSMAN, SELMAN A., and FLORENCE C. TENNEY. Composition of natural organic materials and their decomposition in the soil. III. The influence of nature of plant upon the rapidity of its decomposition. Soil Sci. **26**: 155–171. 1928.
17. WAKSMAN, SELMAN A., FLORENCE C. TENNEY, and ROBERT A. DIEHM. Chemical and microbiological principles underlying the transformation of organic matter in the preparation of artificial manures. Jour. Amer. Soc. Agron. **21**: 533–546. 1929.

EFFECT OF LIGHT AND OF ETHYLENE CHLORHYDRIN ON THE CITRIC ACID CONTENT OF BRYOPHYLLUM LEAVES[1]

JOHN D. GUTHRIE

It has been found that the decrease in acidity that occurs in potato tubers following treatment with ethylene chlorhydrin is due mainly to a decrease in citric acid (4). Evidence has been presented that citric acid is converted to carbon dioxide during the rapid respiration that follows treatment with ethylene chlorhydrin (8). Since these results indicated that citric acid may play a more important rôle in plant metabolism than hitherto realized, it was thought likely that citric acid changes would be found in other cases, especially where changes in acidity occurred. It was also of interest to find whether treatment with ethylene chlorhydrin would bring about a decrease in citric acid in a plant other than potato. Since the diurnal change in the acidity of succulent plants has been investigated more thoroughly than any other acidity change in plants, *Bryophyllum* was chosen for the investigation.

In 1815 Benjamin Heyne (7) reported that the leaves of *Bryophyllum* tasted acid early in the morning, but were tasteless in the afternoon. Since then, the acidity change in succulent plants has been investigated by many workers. It seems to be generally accepted that malic acid is formed from sugars during the night and that the malic acid disappears the next day under the action of light, being either built up into carbohydrates, or broken down into carbon dioxide which is then used in photosynthesis. A detailed review of the subject will be found in the papers of Bennet-Clark (1). Gustafson has studied the diurnal acidity change in *Bryophyllum* in detail (3). Especially good evidence for the connection between the acidity change and carbohydrate metabolism has been given by Wolf (10).

The results of the present investigation show that citric acid as well as malic acid takes part in the diurnal acidity change of *Bryophyllum*. A preliminary report of this has been made (5). A place will therefore have to be found for citric acid in the theories concerning the acid-carbohydrate metabolism of succulent plants. The experiments also show that treatment of *Bryophyllum* leaves in the dark with ethylene chlorhydrin brings about a decrease in acidity, accompanied by a decrease in citric acid. It is interesting that the action of light and of ethylene chlorhydrin on the citric acid content of *Bryophyllum* is similar and suggests the possibility that light acts to stimulate the respiratory process in *Bryophyllum*.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 126.

EFFECT OF LIGHT

The experimental procedure was to express the sap from alternate leaves of *Bryophyllum pinnatum* Kurz. (*B. calycinum*) at mid-afternoon on a bright sunny day. This sample of juice was called the light sample. The plants were then taken from the greenhouse to the dark room and the next morning the juice was expressed from the remaining leaves. This sample of juice was called the dark sample. A pH determination was made on the fresh juice. The juice was then boiled, cooled, water added to the original volume, filtered through cotton, and preserved by shaking with toluene. Titration curves were made on aliquots of the juice, using the quinhydrone electrode and a stream of nitrogen for stirring. Citric acid was determined on duplicate samples of the juice by the official pentabromacetone method of Hartmann and Hillig (6). The conclusions of the present paper of course depend on the validity of this method.[2] Three experiments were made, the details of which were as follows.

TABLE I

EFFECT OF LIGHT ON THE ACIDITY AND ON THE CITRIC ACID CONTENT OF BYROPHYLLUM LEAVES

Experiment No.	pH fresh juice		Titratable acidity; cc. N/10 per 100 cc.			Total acidity; cc N/10 per 100 cc.			Citric acid; cc. N/10 (M/30) per 100 cc.		
	Light	Dark	Light	Dark	Change	Light	Dark	Change	Light	Dark	Change
1	5.51	4.04	26	191	165	186	326	140	10.4	48.9	38.5
2	5.66	3.83	21	199	178	173	333	160	7.1	46.1	39.0
3	5.85	4.00	15	170	155	177	310	133	6.4	34.6	28.2

Experiment No. 1. August 27, 1934; a bright, cloudless day. Juice was expressed from alternate leaves at 1:45 P.M. eastern standard time. The plants were then placed in a dark room until 8 A.M. the next day, when juice was expressed from the remaining leaves.

Experiment No. 2. August 30, 1934; a bright, cloudless day. Procedure was the same as in experiment No. 1. The light sample was taken at 3 P.M. and the dark sample at 8:30 A.M. the next day.

Experiment No. 3. September 11, 1934; clear with a few light clouds. Procedure the same as experiment No. 1. The light sample was taken at 2:15 P.M. and the dark sample at 7:45 A.M. the next day.

The titration curves obtained in experiment No. 1 are given in Figure 1. Similar curves were obtained in the other light experiments. The lowering of the buffer action of the juice due to light is quite large. Since Van Slyke and Palmer (9) have found that the amount of acid or base necessary to

[2] Borgström (2) has recently reported that no citric acid could be detected in the Crassulaceae, using the citric acid dehydrogenase of cucumber seeds. He does not give the species used nor the time of day the samples were taken.

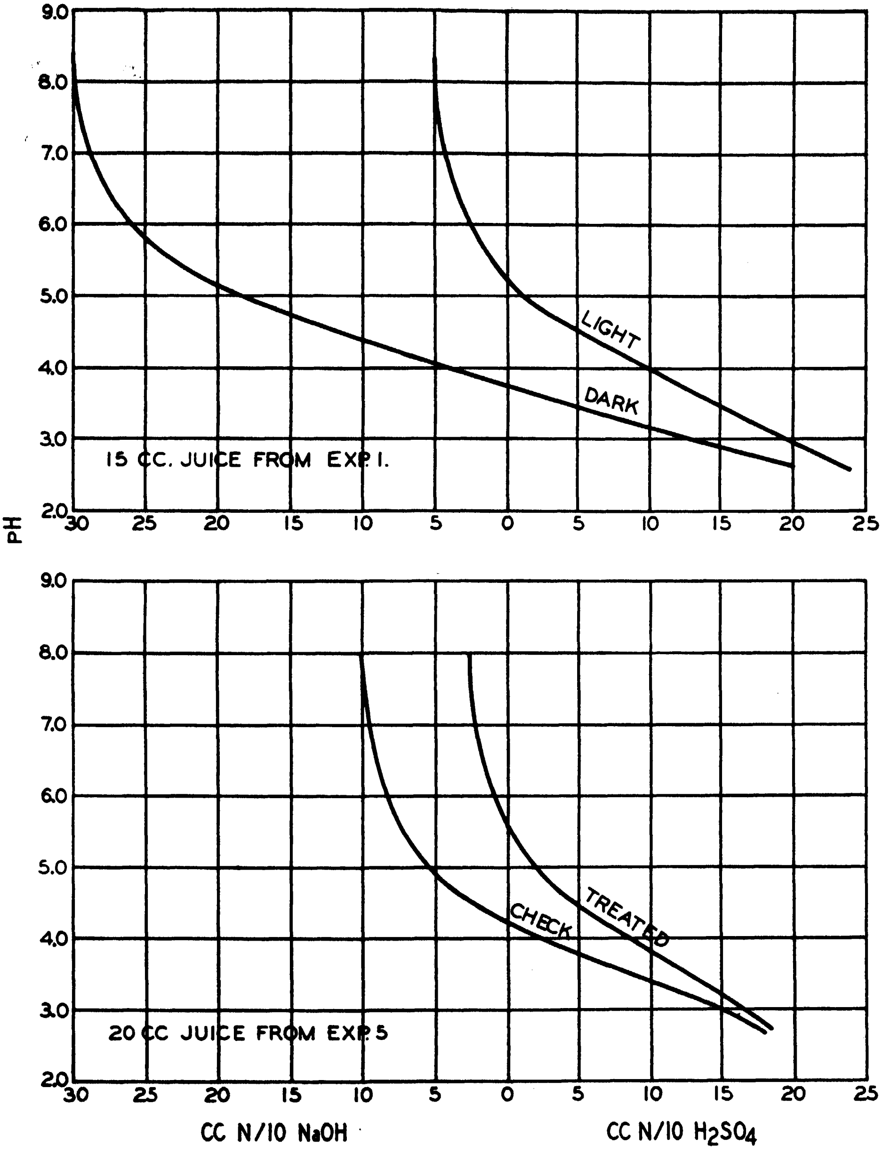


FIGURE 1. The similarity between the effect of light and of ethylene chlorhydrin on the titration curves of the juice of *Bryophyllum* leaves.

change a solution from pH 2.7 to pH 8.0 is a good measure of the total organic acid content, this value was taken from the titration curves and calculated to 100 cc. of juice. The value for titratable acidity was obtained by finding from the curves the amount of N/10 NaOH required to bring the juice to pH 7.0 and calculating to 100 cc. of juice.

The values for pH, titratable acidity, total acidity, and citric acid are given in Table I. The data show that the pH is about 5.7 in the afternoon and changes to about pH 3.9 during the night. There is also a change in titratable acidity and total acidity amounting to about 150 cc. of N/10 per 100 cc. The citric acid content is 5 to 7 times as high in the dark samples as in the light samples. The actual change in citric acid amounts to about 35 cc. of N/10 per 100 cc. The citric acid change, therefore, accounts for about one-fourth of the change in total acids.

EFFECT OF ETHYLENE CHLORHYDRIN

Since it was expected that the effect of ethylene chlorhydrin would be to decrease the content of citric acid, detached leaves were placed in the dark for one or two days before treatment. They were kept in the dark during treatment and until sampled. Preliminary experiments were made using different amounts of ethylene chlorhydrin in order to find the proper concentration. In these experiments the action of the treatments was followed by pH determinations. These experiments showed that little change in pH had occurred two days after the beginning of treatment, but 4 to 6 days after the beginning of treatment a large increase in pH had taken place. The treatment chosen for the experiments was 5 cc. of 8 per cent ethylene chlorhydrin in 11 liters for 24 hours. This was strong enough to bring about a large pH change, but well below the injury point. Three experiments were made, the details of which were as follows.

Experiment No. 4. March 21, 1935; leaves that had been stored for two days in moist chambers in the dark room were used. One-half of these leaves were piled loosely in an 11-liter container and treated with 5 cc. of an 8 per cent solution of ethylene chlorhydrin which was poured on absorbent cotton. The remaining leaves were placed in a similar container for 24 hours. Both samples were then kept in moist chambers until four days after the beginning of the treatment, when the juice was expressed.

Experiment No. 5. March 28, 1935; same procedure as for experiment No. 4 except that the juice was expressed five days after the beginning of the treatment.

Experiment No. 6. May 3, 1935; same procedure as for experiment No. 4 except that the leaves had been in the dark room for only one day before they were treated. At this stage the juice had a pH of 3.99, a titratable acidity of 148 cc., a total acidity of 294 cc., and a citric acid content of 39.0 cc. N/10 per 100 cc. of juice. The treated and check samples were taken 6 days after the beginning of treatment.

The titration curves obtained in experiment No. 5, which are similar to the curves obtained in the other experiments, are given in Figure 1. The ethylene chlorhydrin treatment decreased the buffer capacity of the juice, acting similarly to light in this respect. The values for pH, titratable acidity, total acidity, and citric acid are given in Table II. The ethylene chlorhydrin treatments changed the pH from about pH 4.5 to pH 5.6. The decrease in titratable and total acidity was 40 to 60 cc. N/10 acid per 100 cc. while the decrease in citric acid was about 24 cc. N/100 acid per 100 cc.

TABLE II

EFFECT OF ETHYLENE CHLORHYDRIN ON THE ACIDITY AND ON THE CITRIC ACID CONTENT OF BRYOPHYLLUM LEAVES

Experiment No.	pH fresh juice		Titratable acidity; cc. N/10 per 100 cc.			Total acidity; cc. N/10 per 100 cc.			Citric acid; cc. N/10 (M/30) per 100 cc.		
	Treated	Check	Treated	Check	Change	Treated	Check	Change	Treated	Check	Change
4	5.71	4.44	6	58	52	103	147	44	2.0	26.8	24.8
5	5.87	4.53	9	47	38	106	142	36	4.3	27.0	22.7
6	5.34	4.36	32	92	60	200	257	57	17.6	42.2	24.6

The decrease in citric acid therefore accounts for about one-half of the acidity change brought about by ethylene chlorhydrin treatments. The lower values for total acid in the untreated samples as compared with the untreated samples of the light experiment are probably due for the most part to the time of year and light conditions under which the experiments were made. Bennet-Clark (1, p. 64) has shown that the acidity of the dark samples of succulents is much higher during the summer than during the winter.

SUMMARY

1. There is a five-fold increase in the citric acid content of the juice of *Bryophyllum* leaves during the night. Citric acid accounts for about one-fourth of the diurnal change in acidity.

2. Ethylene chlorhydrin treatments bring about an increase in pH, a decrease in titratable and total acidity, and a decrease in citric acid in the juice of *Bryophyllum* leaves kept in the dark. The decrease in citric acid accounts for about one-half of the change in total acidity.

3. Citric acid as well as malic acid must be taken into consideration in explanations of the acid metabolism of *Bryophyllum* and perhaps of other succulent plants.

LITERATURE CITED

1. BENNET-CLARK, T. A. The rôle of the organic acids in plant metabolism. New Phytol. **32**: 37–71, 128–161, 197–230. 1933.
2. BORGSTRÖM, GEORG A. Further notes on the occurrence of citrate in succulent plants. Preliminary report. K. Fysiograf. Sällskapets i Lund. Förhandl. **4**: 235–243. 1934.

3. GUSTAFSON, FELIX G. Diurnal changes in the acidity of *Bryophyllum calycinum*. Jour. Gen. Physiol. 7: 719–728. 1925.
4. GUTHRIE, JOHN D. Metabolism of citric, sulphuric, and nitric acid in the potato tuber. An explanation for the high pH of the juice of tubers treated with ethylene chlorhydrin. Contrib. Boyce Thompson Inst. 6: 247–268. 1934.
5. ——— Diurnal change in the citric acid content of the leaves of Bryophyllum calycinum. Amer. Jour. Bot. 21: 706. 1934.
6. HARTMANN, B. G., and F. HILLIG. Determination of citric acid in fruits and fruit products. Jour. Assoc. Off. Agric. Chem. 13: 99–103. 1930.
7. HEYNE, BENJAMIN. On the deoxidation of the leaves of *Cotyledon calycina*. Trans. Linn. Soc. [London] 11: 213–215. 1815.
8. MILLER, LAWRENCE P., JOHN D. GUTHRIE, and F. E. DENNY. Induced changes in respiration rates and time relations in the changes in internal factors. Contrib. Boyce Thompson Inst. 8: 41–61. 1936.
9. VAN SLYKE, DONALD D., and W. W. PALMER. Studies on acidosis. XVI. The titration of organic acids in urine. Jour. Biol. Chem. 41: 567–585. 1920.
10. WOLF, JOHANNES. Beitrag zur Kenntnis des Säurestoffwechsels succulenter Crassulaceen. Planta 15: 572–644. 1931.

A RAPID METHOD FOR DETERMINING THE GERMINATIVE POWER OF PEACH SEEDS

FLORENCE FLEMION

Since many peach seedlings (*Prunus persica* (L.) Stokes) are grown commercially each year, it is desirable that a rapid method for testing the viability of peach seeds be available. Because peach seeds have a dormant period necessitating after-ripening at low temperature, ordinary methods of germination require a considerable period of time. Thus intact seeds germinate poorly and irregularly from 14 to 26 weeks after being placed in a moist medium at 5° or 10° C. (but not at 15° or 20° C.), while 10 to 14 weeks at 5° or 10° C. are required if the hard pericarp is removed before the seeds are placed at low temperature (1). Recent experiments (2) have shown that if both the pericarp and the inner brown coat are removed and the seeds are mixed with moist peat moss and kept at 25° C., germination occurs within five to seven days. Further experiments to be reported in this paper have shown that when the percentage germination thus obtained is compared with that resulting when the seeds are after-ripened at 5° to 10° C. for three to four months the data obtained by the two methods show good agreement. A simplified method is therefore available for a rapid and easy determination of the vitality of peach seeds before planting in the fall.

MATERIALS AND METHODS

Peach seeds were obtained from various orchards and canning factories in New York, Pennsylvania, North Carolina, and Arkansas. The pits were freed of pulp by the use of a Hobart mixer, washed, and spread out to dry. They were then stored dry at room temperature and samples taken when the tests were to be made.

For the removal of the hard pericarp a wedge and a hammer can conveniently be used. When large numbers of seeds are to be handled it may be desirable to construct a machine as illustrated in Figure 1 C. This consists of a wedge fastened to a plunger which is attached to a lever. The lever operates an eccentric shaft so that the plunger breaks open the pericarp at the lower point of eccentricity but is mechanically prevented from from being lowered far enough to crush the embryo. By adjusting the length of the plunger and in some cases the type of wedge, the machine can also be used for breaking the outer coats of seeds of species other than peach.

When the pericarp had been removed the seeds were soaked in water overnight at room temperature after which the inner coats were carefully

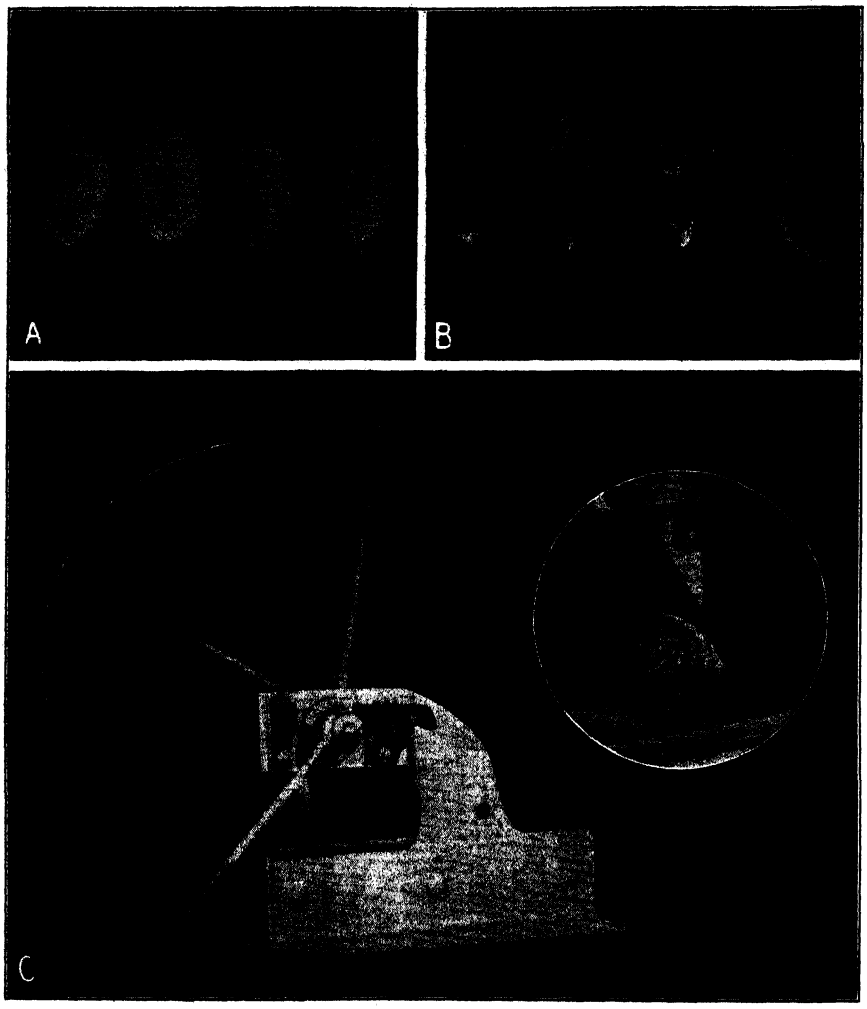


FIGURE 1. A. Non-after-ripened embryos germinating at room temperature; B. After-ripened seeds germinating at low temperature. C. Machine used for cracking peach pits.

peeled off. This could be done most readily if a hole was first made in the coat by pricking with a needle. It is necessary to be careful in removing the coats since injury to the embryo is to be avoided. Injured embryos are quickly attacked by organisms and thus fail to germinate.

Moist granulated peat moss was found superior to other media such as sand or soil for it holds a large reserve of water and an adequate supply of air. The peat was moistened and wrung out thoroughly by hand until it crumbled apart readily. The seeds were washed in water, mixed in with the slightly damp peat, put in containers, and placed at the proper temperatures.

TABLE I

COMPARISON OF THE TWO METHODS OF TESTING THE GERMINATIVE POWER OF PEACH SEEDS

Source of seed	Test started days after receipt*	10 days at 25° C.		After-ripened at 5° C.	% germination	
		No. of embryos used	Per cent germination	No. of seeds used	After 3 months	After 4 months
North Carolina 1933	8	210	62	250	56	60
	94	205	72	100	46	60
	142	37	53	75	52	54
	228	100	68	75	48	49
	321	119	65	100	56	57
	815	110	37	100	19	27
	142*	75	77	75	78	79
	228*	70	64	100	55	55
	321*	109	65	100	68	70
Arkansas 1934 (Elberta)	0	315	93	150	100	100
	128	435	82	100	86	89
	277	162	70	100	74	74
	524	145	36	130	61	61
Illinois 1934 (Champion)	0	30	83	30	90	90
Pennsylvania 1935 (Elberta)	45	100	94	100	63	65
North Carolina 1932	1145	100	0	100	0	0

* All material stored at room temperature from time of receipt to start of experiments except material marked thus which was stored at 5° C. for number of days indicated.

In five to seven days at room temperature (20° to 25° C.) the viable seeds showed hypocotyl development as illustrated in Figure 1 A. The material was examined daily and germinated seeds removed and counted. At the same time non-viable seeds showing obvious evidence of deterioration were also removed and counted. All the embryos were accounted for in this way in about ten days. The results obtained in such a test were compared with percentage germination obtained when comparable seeds with only the pericarp removed were placed in moist peat moss at 5° C.

Under these conditions, germination (Fig. 1 B) takes place from two to four months after the start of the low temperature period.

RESULTS

The results obtained in tests with seeds from a number of different sources and from crops of different years are given in Table I. By comparing the figures for percentage germination in columns 4 and 7 it can be seen that the rapid method of testing gave results in satisfactory agreement with those obtained when the seeds are after-ripened at low temperature for the germination test.

DISCUSSION

Recently, Tukey and Barrett (3) have published a method for the rapid detection of the germinative power of peach seeds. Their method involves the placing of naked embryos in an agar medium with nutrient materials under sterile conditions. Results are obtained in seven days. According to our experiments the presence of neither the agar nor the nutrient materials is necessary, for the naked embryos will give a satisfactory germination test if mixed with damp peat moss. The procedure described in the present paper is thus much simpler and requires no apparatus or material not readily available to commercial growers.

In a previous publication (2) it was shown that the dormant seeds of peach as well as of a number of other species can be made to produce plants when the naked embryos are placed at room temperature in moist peat moss. The plants resulting showed abnormal growth which was described as dwarf-like in character and which persisted for a number of months before apparently normal growth began. The procedure described in the present paper is recommended for use as a method of testing vitality only and not necessarily as a method for producing peach seedlings since seedlings resulting would exhibit the dwarfishness referred to above. Von Veh (4), however, has published several papers in which he recommends the commercial production of seedlings of apple, pear, quince, plum, and cherry from the non-after-ripened seed by using excised embryos.

Perfectly normal seedlings of peach can, however, be readily obtained by subjecting the seeds to a period at 5° to 10° C. For maximum seedling production, the seeds with pericarps removed should be mixed in moist peat moss and placed in a refrigerator maintained between 5° to 10° C. (40° to 50° F.). Since the plantings are to be made in April, seeds should be placed at low temperature in late January. The material should be examined about every two weeks and water added if necessary. As soon as germination begins, the whole lot can be planted and placed in a cool frame out-of-doors.

SUMMARY

If both the pericarp and the inner seed coat are removed and the naked embryos mixed with moist granulated peat moss at room temperature a test of the germination capacity of dormant peach seeds can be obtained in ten days. The percentage germination thus obtained agrees well with that obtained when the seeds are after-ripened at 5° to 10° C. for the germination test. This method requires no special equipment and can be carried out readily under commercial conditions.

LITERATURE CITED

1. CROCKER, WILLIAM. Dormancy in hybrid seeds. Mem. Hort. Soc. New York **3**: 33–38. 1927. (*Also in* Boyce Thompson Inst. Prof. Pap. 1(6): 36–41. 1927.)
2. FLEMION, FLORENCE. Dwarf seedlings from non-after-ripened embryos of peach, apple, and hawthorn. Contrib. Boyce Thompson Inst. **6**: 205–209. 1934.
3. TUKEY, H. B., and M. S. BARRETT. Approximate germination test for non-after-ripened peach seed. Plant Physiol. **11**: 629–633. 1936.
4. VEH, ROBERT VON. Eine neue Methode der Anzucht von Sämlingen, unabhängig von Ruheperioden und Jahreszeit (bei Äpfeln, Birnen, Quitten, Pflaumen, Kirschen). Züchter **8**: 145–151. 1936.

ISOLATION OF CITRIC ACID FROM POTATO TUBERS[1]

JOHN D. GUTHRIE

Evidence that citric acid plays an important part in the respiration of potato tubers has been given in previous papers (4, 6). This conclusion was reached on the basis of data obtained by the pentabromacetone method. Although this method is considered specific for citric acid, it was thought important to obtain positive proof of the presence of citric acid in potato tubers. No such proof could be found in the literature. Baup (1) in 1835 stated that citric acid was present in potato tubers in commercial quantities, but gave no evidence to support his statement. Michaelis (5) in 1838 detected citric acid in the lead acetate precipitate of an extract of potato tubers, but did not state what test was used and makes no mention of having the pure acid. Therefore, the isolation of citric acid from potato tubers was undertaken. After trying several procedures, the following was found to give good results.

Juice was expressed from potato tubers (*Solanum tuberosum* L. var. Irish Cobbler) and the starch centrifuged out. The juice was then boiled, cooled, and filtered to remove proteins. Eight hundred cc. of the boiled, filtered juice were mixed with 25 cc. of 20 per cent hydrochloric acid, 200 cc. water, and 2.5 liters of alcohol. After standing one hour a small coagulum was filtered out and 200 cc. of 10 per cent barium acetate were added to the filtrate. After standing overnight the precipitate was separated by decantation and centrifuging. It was washed once with 70 per cent alcohol, taken up in 25 cc. of 20 per cent hydrochloric acid, heated on the steam bath, and filtered to remove barium sulphate derived from sulphate present in the juice. The filtrate was almost neutralized with 20 per cent sodium hydroxide, heated on the steam bath, allowed to stand several hours, the precipitate filtered out, and washed with water. The precipitate was taken up in 10 cc. of 20 per cent hydrochloric acid and a small coagulum filtered out. The filtrate was almost neutralized with normal sodium hydroxide, digested for one-half hour on the steam bath, and filtered while hot. The precipitate was digested on the steam bath with water, filtered hot, washed with water, and dried. It weighed 5.1 g.

This precipitate was ground in a mortar with 30 cc. of normal sulphuric acid and then digested in a boiling water bath for 20 minutes. After cooling it was filtered and the barium sulphate washed with water. The filtrate was evaporated on the steam bath and then in a vacuum desiccator. The sirupy residue was extracted repeatedly with small portions of ethyl ether

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 127.

at its boiling point until no further material dissolved. The ether filtrate was evaporated to dryness and the crystalline residue was washed with ether. It weighed 0.81 g. It was recrystallized from ether and 0.50 g. was obtained in the first fraction. It was dried at 103° C.

The material melted at 152° C. Beilstein (2, p. 559) gives 153° C. as the melting point of citric acid. It crystallized from water on evaporation on a microscope slide in elongated six-sided plates showing parallel extinction. The angle adjacent to the long side was about 117°. Refractive indices were 1.51 and 1.54. In these respects it was identical with a known sample of citric acid, crystallized under the same conditions, and different from malic acid, which crystallized in needles showing oblique extinction. The acid equivalent was determined by micro titration. The substance, 5.38 mg., neutralized 8.35 cc. N/100 sodium hydroxide. Found: acid equivalent 64.4; calculated for anhydrous citric acid 64.0. The para-phenylphenacyl ester was prepared by the procedure of Drake and Bronitsky (3). It was recrystallized from benzene. Melting point 146.5° C. A preparation from a known sample of citric acid melted at 145° C. The mixed melting point was 145.5° C. Drake and Bronitsky give 146° C. as the melting point of the para-phenylphenacyl ester of citric acid.

Professor Herbert K. Alber of New York University determined carbon and hydrogen by the micro-combustion method. The substance, 5.779 mg., gave 2.15 mg. H_2O and 7.99 mg. CO_2. The substance, 5.311 mg., gave 1.95 mg. H_2O and 7.335 mg. CO_2. Found: carbon 37.71, 37.67 per cent; hydrogen 4.16, 4.11 per cent. Calculated for $C_6H_8O_7$, carbon 37.41 per cent, hydrogen 4.20 per cent.

SUMMARY

Citric acid was isolated from potato tubers.

LITERATURE CITED

1. BAUP. Über Bestandtheile der Kartoffelpflanze (*Solanum tuberosum*). Repert. Pharm. **53**: 390–393. 1835.
2. Beilsteins Handbuch der organischen Chemie. 4 Aufl. Bd. 3. 938 pp. Julius Springer, Berlin. 1921.
3. DRAKE, NATHAN L., and JACK BRONITSKY. Para-phenylphenacyl bromide, a reagent for identifying organic acids. Jour. Amer. Chem. Soc. **52**: 3715–3720. 1930.
4. GUTHRIE, JOHN D. Metabolism of citric, sulphuric, and nitric acid in the potato tuber. An explanation for the high pH of the juice of tubers treated with ethylene chlorhydrin. Contrib. Boyce Thompson Inst. **6**: 247–268. 1934.
5. MICHAELIS, FR. Chemische Untersuchung einer vermeintlich schädlichen rothen Karttoffelart. Arch. Pharm. **63**: 233–247. 1838.
6. MILLER, LAWRENCE P., JOHN D. GUTHRIE, and F. E. DENNY. Induced changes in respiration rates and time relations in the changes in internal factors. Contrib. Boyce Thompson Inst. **8**: 41–61. 1936.

GERMINATION AND SEEDLING PRODUCTION IN LILIUM SP.

LELA V. BARTON

INTRODUCTION

In spite of the fact that it is becoming increasingly popular to grow lilies from seeds, no systematic work seems to have been done on the germination behavior of the various forms. From the lily fancier's point of view, many advantages accrue with the home production of lily bulbs. Bulbs, domestic or imported, are apt to be inferior in vigor, and may carry the dread lily mosaic which is one of the great hindrances to successful lily culture. Seeds do not carry the mosaic disease, and seedling bulbs have the advantage of an early and complete adaptation to the environment in which they are expected to grow. On the other hand, it takes much longer to bring the plants to the flowering stage when they are grown from seed. This is especially true of some slow-growing varieties.

A large number of short popular articles (2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 18) have appeared from time to time in horticultural or floricultural magazines reporting experiences of various lily growers as to seed germination. Many of the reports are for seeds which germinate without difficulty, such as *Lilium philippenense*, *L. regale*, *L. tenuifolium*, *L. henryi*, and *L. willmottiae*. A number, however, contain notes to the effect that in some cases, lily seeds do not appear above ground the first season but produce small bulbs. These authors vary in their recommendations for fall and spring planting.

More detailed reports have appeared in a few instances. Three hundred and eighty-two different plantings were made by Stout (17). He generally obtained excellent results when seeds were sown as soon as mature, or kept dry at room temperature and planted in the spring following collection. He pointed out that in certain plantings, seeds remained dormant for several months, even over summer, when other plantings of the same species handled in the same way gave quick germination. He found two main habits of germination and subsequent growth of seedlings: (1) cotyledons extend above ground and become green, and (2) cotyledons remain in the soil. Of the latter, one group sends up the first true leaf soon after germination, while in a second group, the first leaf does not appear above ground until a year later. *L. superbum* and *L. canadense* he placed in the second group.

Stern (16) in 1933 classified germination of lily seeds in essentially the same way as Stout. Among those species he included in the second group,

with germination underground, were *japonicum* and *canadense*, and he quoted others as including *superbum* and *auratum* in this class.

Slate (15) divided lilies in three groups as regards germination behavior. The first two groups were comparable to those of Stout (17) and Stern (16). To these he added a third or "erratic" group, whose behavior varied (*candidum*, *henryi*, *testaceum*, *rubellum*, and *croceum*). He included *auratum*, *canadense*, *japonicum*, and *superbum* in the second group, or those that germinate and remain underground the first season. Slate suggested that if seeds of this second group could be planted sufficiently early in the fall, they should germinate underground, go through their rest period during the winter and start off on the second season in the spring. However, since it is often difficult to secure seeds of most lilies early enough, he preferred to plant these second year lilies in the spring.

Ingram (11) in 1935 reported similar effects for *L. auratum platyphyllum*, and *L. martagon album superbum*. He did not sow seeds of the former in the autumn but when sown outside in April, germination occurred in July and first leaves during January. For *martagon album superbum* also spring sowing was effective, the majority of the true leaves appearing the following March. In addition, he sowed seeds of this variety in a cold frame in September soon after ripening, and reported that they appeared to have germinated the same autumn while it was still warm because all of them came up the next April.

These facts might account for the report by Taylor (19) in 1932 that *L. auratum* sowed outside in the fall gave good seedling production in the spring or even as early as February. A warm autumn would make these results possible.

Growers are not agreed as to the importance of fresh seeds for good germination. Grove (10) stated that *L. giganteum* seeds should be sown as soon as ripe. In another report (9) he remarked that a fine harvest season had a stimulating effect on seed germination, probably due to the maturity of the seeds. According to Suttle (18) it is a waste of time to try lily seeds carried over from one season to another. Taylor (19) warned against using imported seed of *Lilium japonicum*, *L. auratum* and all its sub-species, and possibly *L. rubellum*. He said (p. 76), "The seed may lie dormant for two years in the soil, everything depending upon how long the seed has been kept." "Home-saved" seed of all of these if sown in autumn, he found, would come through in abundance in the spring.

Stern (16) experimented for a number of years trying to find out whether old seed took a longer time for germination than fresh seed. Statement of his conclusions was indefinite. The time of sowing makes no difference according to Weston (20), while Craig (3) found that *L. auratum* started better from year-old than from fresh seeds.

None of the growers mentioned above seemed to have experienced any

difficulty in growing lily seedlings to the flowering stage, once germination had taken place, although a few forms were reported as slow growers. Weston (21), in December 1934, challenged the advisability of growing from seeds. He tried large lots of *L. candidum* seed over a seven- or eight-year period, but his seedlings never reached the stem-making size. *L. giganteum* was sown "by the ounce" but none got beyond the two-leaved stage. He was not able to get *martagon* to flower.

Experiments have been carried out at this laboratory to trace the progress of germination and leaf formation in some of the lilies. *L. auratum* Lindl., *L. canadense* L., *L. concolor* Salisb., *L. japonicum* Thunb., *L. rubellum* Baker, *L. superbum* L., and *L. szovitsianum* Fisch & Ave-Lall. have been used for this study. Preliminary tests on the effect of the age of the seed are reported.

Samples of all seedling lots have been grown and the plants and flowers identified as reported.

This project has been carried out at the suggestion and with the cooperation of Mr. Keith O'Leary, Fellow of Lily Disease Investigation, through whom the seeds were obtained and who grew and identified the plants. *L. auratum* collections were made from plants growing on the Institute farm at Yonkers, New York, while other seeds were obtained from Japan.

METHOD

The experiments performed may be divided into two general classes: (1) germination at various controlled temperatures, and (2) seedling production.

Germination tests were made over a wide range of temperatures for *L. auratum*, *L. canadense*, *L. concolor*, *L. japonicum*, *L. rubellum*, and *L. superbum*. The seeds were mixed with moist granulated peat moss and placed at controlled temperatures. As the hypocotyls appeared, the seedlings were removed and planted in soil in pots. Since such seedlings failed to produce shoots when transferred to pots in the greenhouse, a series of tests were made where seedlings in pots were given various temperature treatments for different lengths of time in an effort to break the epicotyl dormancy which apparently existed in some of the seeds. These pots were later transferred to greenhouses at temperatures of 15° C. or 21° C.

For seedling production in flat plantings, a greenhouse at 21° C., a cold room at 10° C., and the cold frames either open, board-covered, or mulched and board-covered during the winter were used. The soil mixture for all pot and flat plantings was made of equal parts of sand, sod soil, and granulated peat moss.

"Germination" counts were made when the hypocotyl broke through the seed coat while "seedling production" counts were made with the appearance of the cotyledons or first leaves above ground.

RESULTS

LILIUM AURATUM

Germination

Temperature effects. Since a number of plants were available from which seeds could be collected, the most extensive tests were made with this species. Preliminary tests with small quantities of seeds were made in 1931 and 1932. In 1933 and 1934 large quantities of seeds were collected. In 1933, collections were made on September 7 and 16, October 11 and 23, and November 3, 13, 17, and 23. In 1934, all seeds were collected on

TABLE I

GERMINATION OF L. AURATUM SEEDS IN PEAT AT VARIOUS TEMPERATURES

Crop	Collection		Per cent germination at various temperatures ° C.								
	Date	Condition of seed	15	20	25	30	35	10 to 20*	10 to 30*	15 to 30*	20 to 30*
1933	Sept. 7	Green and soft embryos appeared full size	0	0	—	—	—	—	—	0	—
	Sept. 16	Green and soft embryos appeared full size	—	0	—	—	—	—	—	0	—
	Oct. 11	Half brown, half white	51	57	—	—	—	—	—	37	—
	Oct. 23	Half brown, half white rather small endosperm	—	18	—	5	—	2	—	22	—
	Nov. 3	Light brown—moist	—	88	—	0	—	0	—	1	—
	Nov. 13	Dark brown	16	42	0	0	0	2	1	7	12
	Nov. 17	Light brown—fairly dry	28	72	2	0	0	1	4	7	1
	Nov. 23	Light brown	26	55	0	0	0	1	1	6	3
1934	Nov. 14	Dark brown—moist	16	85	1	0	1	3	2	17	2
	Nov. 14	Dark brown—dry	19	70	5	0	—	1	0	3	0

* Daily alternation. At lower temperatures 16 hours and at higher temperatures 8 hours daily.

November 14. Since frost occurs in this region before the capsules are mature, the fruit stalks were cut about the middle of October and placed in large jars of water in a greenhouse at 21° C. where the seeds matured and final collections were made.

Results of germination tests are shown in Table I. It will be noted that green, soft seeds collected on September 7 or 16 failed to germinate. By October 11, the seeds were beginning to turn brown and germinated to 57 per cent at 20° C. The best germination from the 1933 crop was obtained from collections made between November 3 and 17. A collection on November 14, 1934, was also good whether the capsules were moist or dry at the time of collection. For five different seed lots a wide range of constant temperatures as well as four daily alternating temperatures were used. However, the range effective for germination proved narrow, 20° C. being the only temperature with consistently good germination.

Roots began to appear in about six weeks or two months and germination extended rather uniformly over a period of five or six months. Any attempt made to shorten the period of germination failed. The seeds were pre-treated at 1°, 5°, or 10° C., and at 10° C. after previous periods of 1, 2, 3, 4, and 5 months at 20° C. In the last case, germination was always in progress at the time the 20° C. culture was transferred to 10° C. Samples from these low temperatures were taken monthly up to ten months with no subsequent hastening of germination although germination percentages up to 99 resulted at 20° C. Corresponding samples made in the greenhouse showed no seedlings but many of them developed extensive root systems. Further results from these greenhouse samples will be given under "Seedling Production" below.

TABLE II

L. AURATUM. EFFECT OF STORAGE ON GERMINATION OF SEEDS. 1934 CROP

Condition of seed	Months of storage	Per cent germination at 20°C. after sealed storage at various temps. ° C.					
		1	5	10	20	25	30
Moist	3	94	89	83	0*	0*	0*
	5	82	—	27	77**	—***	—***
	12	88	88	44	0†	—	—
Air-dry	3	90	89	88	89	80	87
	5	48	43	58	55	52	41
	12	44	29	38	47	14	7‡

* Very moldy—discarded after 2 months.
** Started germinating after 3 months.
*** Seeds rotten in storage container. Discarded.
† Kept 3 months and then discarded because of mold.
‡ Many rotten.

Storage effects. The 1934 crop, both moist and dry seeds, were stored in bottles sealed with deKhotinsky cement at 1°, 5°, 10°, 20°, 25°, and 30° C. two days after harvest. Germination tests were made in moist granulated peat moss at 20° C. after 3, 5, and 12 months of storage.

Moist seeds stored at 20°, 25°, or 30° C. had become moldy at the end of three months. A test made at this time showed no germination as the seeds rotted and were discarded after two months in germination condition. With seeds from 20° C. storage after five months, the seeds did not rot so soon and it was possible to keep the cultures. Although germination did not begin for three months, 77 per cent germination was obtained (Table II). After 12 months of storage at 1° or 5° C. moist seeds still showed complete retention of vitality while storage at 10° C. was not so effective.

Table II will show that air-dry seeds kept well at all temperatures for three months and equally well at all temperatures after five months, with

a falling off at storage temperatures of 25° or 30° C. after twelve months.

A storage experiment on *Lilium regale* now in progress at this laboratory should shed more light on the question of viability of old lily seeds.

Seedling Production

Oven pre-treatment. Since *L. auratum* germinated well at 20° C. without pre-treatment of any kind, there seemed to be no special problem involved. However, when these seedlings were planted in the greenhouse at 21° C., no shoots appeared above ground. This pointed to a dormancy of the epicotyl.

TABLE III

L. AURATUM. EFFECT OF PRE-TREATMENT OF GERMINATED SEEDS ON SHOOT PRODUCTION IN A GREENHOUSE AT 15° C. FIGURES ARE AVERAGES OF DUPLICATE POTS UNLESS OTHERWISE INDICATED

Crop	Pre-treatment time	Per cent of shoots produced from germinated seeds pre-treated at var. temps.					
		None	1° C.	5° C.	10° C.	Refrigerator	15° C.
1933	None	46	—	—	—	—	—
	1 mo.	—	50*	54*	78*	70	—
	6 wks.	—	60*	14*	63*	—	—
	2 mos.	—	50*	66	71	49	41*
	3 mos.	—	—	—	—	100*	—
1934 (moist)	None	28	—	—	—	—	—
	2 wks.	—	50	43	53	35	25
	1 mo.	—	45	50	60	58	10
	6 wks.	—	88	75	55	73	23
	2 mos.	—	85	93	65	90	20
	3 mos.	—	83	85	83	85	18
1934 (dry)	None	18	—	—	—	—	—
	2 wks.	—	38	23	13	30	5
	1 mo.	—	48	50	58	43	15
	6 wks.	—	68	57	64	70	3
	2 mos.	—	83	88	74	90	20
	3 mos.	—	63	95	100	93	37

* Single pot.

In order to solve this problem, the seedlings were removed from the peat as soon as the hypocotyl had started to grow, and planted in six-inch pots containing the soil mixture described above. As a rule 20 seedlings were planted in each pot, although the number varied from 14 to 35 depending on the number available on that particular date. In most cases, duplicate pots were used for each test. These pots were then placed at 1°, 5°, 10°, or 15° C. or in a refrigerator with a range in temperature of 3° to 10° C. for periods of two weeks to three months. They were then removed to a greenhouse of 15° C. or 21° C., where the appearance of the first leaves was noted. Since control lots planted in the 21° C. greenhouse

did not produce seedlings, the majority of transfers were made to a greenhouse at 15° C. where the untreated control gave some seedling production (Table III). Most of the transfers were made during the winter or early spring when the greenhouse temperature could be controlled. Several were made in May, however, and a few as late as August. The treated seedlings grew equally well regardless of the time of transfer, but untreated controls failed to develop seedlings when planted in April or later.

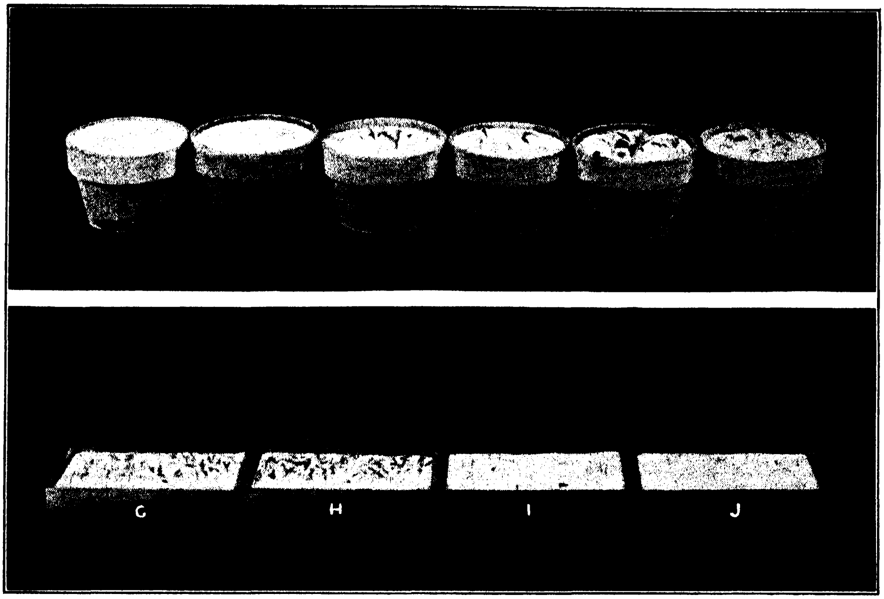


FIGURE 1. (Top row) Epicotyl pre-treatment of *L. auratum* seedlings; 20 seedlings per pot. Photographed Sept. 18, 1935. (A) Untreated control to greenhouse April 23, 1935. (B) Pre-treated 2 weeks at 10° C. to greenhouse April 17, 1935. (C) Pre-treated 1 month at 10° C. to greenhouse April 26, 1935. (D) Pre-treated 6 weeks at 10° C. to greenhouse May 7, 1935. (E) Pre-treated 2 months at 10° C. to greenhouse May 20, 1935. (F) Pre-treated 3 months at 10° C. to greenhouse June 14, 1935. (Bottom row) Effect of spring, summer, and fall plantings on seeds of *L. auratum*. In board-covered frame over winter. Photographed May 31, 1935. Planted 1934. (G) April; (H) June; (I) August; (J) September.

Duplicates transferred to the greenhouse after two weeks or one month or even six weeks at the low temperatures were somewhat erratic in their response. If the pre-treatment were extended to two or three months, however, consistently good results were obtained. The figures in Table III represent averages of duplicates unless otherwise indicated.

The beneficial effect of 1°, 5°, or 10° C. or refrigerator temperature on epicotyl growth is clear from a study of the data. After as short a period as two weeks, the seedling production was increased in most cases. Two

or three months at these temperatures probably overcame the epicotyl dormancy completely. Fifteen degrees C. proved much less effective, although some seedlings resulted from treatment at this temperature. Oven treatment at 15° C. gave results comparable with the untreated controls in a 15° C. greenhouse. The variation in percentage of first leaves obtained in the greenhouse after apparently optimum treatment might have been due to variation in the size and vigor of the young roots at the time of planting in the pots for epicotyl treatment.

TABLE IV

L. AURATUM. EFFECT OF PLANTING IN COLD FRAMES

Crop	Date of planting	Per cent seedling production			
		1935		1936	
		Mulched	Board-covered	Mulched	Board-covered
1933	Nov. 3, 1933	35	90	—	—
	Nov. 18, 1933	86	80	—	—
	Mar. 19, 1934	49	67	—	—
	Apr. 4, 1934	63	79	—	—
	May 4, 1934	48	73	—	—
	June 4, 1934	46	70	—	—
	July 3, 1934	42	68	—	—
	Aug. 3, 1934	8	3	61	54
	Sept. 4, 1934	2	1	48	42
	Oct. 3, 1934	1	1	27	32
1934	Dec. 1, 1934	1	1	73	84
	Jan. 2, 1935	0	1	69	90
	Feb. 1, 1935	0	1	61	77

The top row of Figure 1 shows the effect of pre-treatment at 10° C. for two weeks to three months before transfer to the greenhouse. The control shows no seedlings since the pot planting was made in April. On the other hand, the transfer after three months pre-treatment shows good seedling production in spite of the fact that it was placed in the greenhouse the middle of June, when the temperature was high.

Flat plantings. Seeds of the 1933 crop were sown directly in flats in November 1933 and March, April, May, June, July, August, September, and October 1934. These flats were placed outside in the cold frames. Some of them were covered with both mulch and board cover while others had board cover only during the winter months. Duplicate flats of 300 seeds each were used for each planting except on November 18, 1933, when duplicates of 250 seeds were used. From Table IV it will be seen that there was good seedling production in the board-covered frame in the spring of 1935 if the seeds were planted in November 1933, or any time in 1934 up to August. Seeds planted in August, September, or October, 1934, gave

only occasional seedlings in the spring of 1935 but produced fairly good stands in 1936, the October planting being poorest.

Duplicate flats of 200 seeds each of the 1934 crop planted in December 1934 and January and February 1935 failed to produce seedlings in the spring of 1935 but produced good seedling stands in 1936.

From these flat plantings which include every month of the year, it was shown that the most unfavorable months for planting seeds of *L. auratum* are August, September, and October. It is apparent that the seeds do not require two winters and an intervening summer to produce plants above ground since spring or summer plantings are as effective as late fall or winter planting.

Figure 1, lower row, shows the production of the first leaves of *L. auratum* in May 1935 from plantings made outside in April, June, August, and September 1934.

A protection of mulch under the board cover proved inferior in many cases to board cover alone. This does not mean that the mulch was ineffective. In fact, the higher temperature provided by the mulch (approximately 5° C. as opposed to approximately 3° C.) caused a more rapid growth of the leaves, so that unless careful watch was kept, some of the seedlings were likely to come above the ground and be killed by the mulch. In a number of instances seedlings appeared before the removal of the mulch in the spring. Flats containing such seedlings, many of which had grown long and etiolated, were removed to a greenhouse as soon as the seedlings were discovered. In practice, then, unless the beds can be examined frequently, a board cover without mulch furnishes the best winter protection for flats of lily seeds.

Results from outside plantings were consistent with those of germination and oven pre-treatment of seedlings as reported above.

The seeds required a warm period for germination and establishment of the roots followed by a cold period for breaking the dormancy of the epicotyl so the leaves could emerge above ground. This same requirement for seedling production has been reported for tree peony (Barton 1). Hence if the seeds are planted in the fall or winter, they remain in the soil unaffected as far as germination is concerned until the warmth of the following spring brings about root production. There is probably some harm done by fall planting, especially early fall planting, since a short warm period is insufficient to bring about germination and may cause the seeds to rot.

Moist and dry seeds of the 1934 crop were planted in flats and placed in a 21° C. greenhouse the middle of November 1934. From here they were transferred to a board-covered frame after one, two, and three months. This means that the transfers to the outside took place in the middle of December, January, and February, 1935. Only a few seedlings resulted from these transfers in 1935, while good seedling production was

obtained in 1936. This is not surprising in view of the results reported above. Since root production does not start before six weeks or two months at 20° C. and since the root system must have begun to form before transfer to low temperature in order to have such low temperature effective, one or two months at the high greenhouse temperature was insufficient. On the other hand, three months at high temperature was adequate for the emergence of a number of roots but the subsequent short time at low temperature available outside after the transfer from the greenhouse was insufficient to break the dormancy of the epicotyl.

TABLE V

LILIUM—EFFECT OF TEMPERATURE ON THE GERMINATION OF VARIOUS SPECIES

Species	Crop	Per cent germination at various temperatures °C.							
		15	20	25	30	10 to 20*	10 to 30*	15 to 30*	20 to 30*
Canadense	1933	54	73	19	1	0	0	4	14
	1934	74	88	11	0	1	2	6	0
Japonicum	1933	—	12	0	0	1	0	3	—
	1934	55	76	0	0	2	0	2	0
Rubellum	1934	77	88	2	—	27	—	63	—
Superbum	1933	35	82	6	2	0	0	2	4
	1934	57	78	8	0	0	6	10	1
Concolor Lot 1	1934	40	52	44	7	21	23	38	46
Lot 2	1934	13	79	45	4	26	31	32	34

* Daily alternation. At lower temperatures 16 hours and at higher temperatures 8 hours daily.

This fact was further borne out by the two sample plantings from the low temperature pre-treatment of seeds in the ovens (mentioned above under "Temperature effects on germination") made in September 1935 in soil in the 21° C. greenhouse. Good roots had formed by January 8, 1936 at which time the flat was transferred outside to the board-covered frame where 77 to 100 per cent seedling production was obtained by April 1936.

OTHER SPECIES

Of the other species with which fairly extensive tests were made, *L. concolor* proved to be of the type which germinates and produces shoots readily. The roots appeared in a short time and the cotyledons came directly through the soil without any pre-treatment.

L. canadense, *L. japonicum*, *L. rubellum*, and *L. superbum*, however, showed the same type of germination requirement as *L. auratum*. This was likewise true of *L. szovitsianum* although the seed supply in this case was limited.

Table V which shows germination or root production at various temperatures for five of these species indicates 20° C. as effective although some of these forms, especially *L. concolor*, tolerate a wider range in germination temperature than *L. auratum*.

TABLE VI

LILIUM SP. 1934 CROP. EFFECT OF PRE-TREATMENT OF GERMINATED SEEDS ON SHOOT PRODUCTION IN A GREENHOUSE AT 15° C. FIGURES ARE AVERAGES OF DUPLICATE POTS UNLESS OTHERWISE INDICATED

Species	Pre-treatment time	Per cent of shoots produced from germinated seeds pre-treated at var. temps.					
		None	1° C.	5° C.	10° C.	Refrigerator	15° C.
Canadense	None	15	—	—	—	—	—
	2 wks.	—	3	8	15	8	10
	1 mo.	—	38	23	30	20	0
	6 wks.	—	55*	58	40	50	3
	2 mos.	—	63	78	65	78	20
	3 mos.	—	88	90	85	90	8
Japonicum	None	5	—	—	—	—	—
	2 wks.	—	13	0	0	5*	—
	1 mo.	—	13	0	8	0	—
	6 wks.	—	13	3	15	15*	0*
	2 mos.	—	30	8	3	40*	0*
	3 mos.	—	35	25	50	55*	—
Rubellum	None	0	—	—	—	—	—
	2 wks.	—	15	25	8	10	0*
	1 mo.	—	23	18	23	25	0
	6 wks.	—	38	33	40	39	3
	2 mos.	—	48	50	45	25	3
	3 mos.	—	68	65	80	85	3
Superbum	None	0*	—	—	—	—	—
	2 wks.	—	0*	0*	0*	0*	—
	1 mo.	—	10*	5*	0*	0*	—
	6 wks.	—	15*	10*	0*	6*	—
	2 mos.	—	77*	70*	40*	80*	—
	3 mos.	—	70*	100*	83*	80*	0*
Szovitsianum	None	0	—	—	—	—	—
	1 mo.	—	—	20*	—	—	—
	6 wks.	—	5*	0*	5*	—	—
	2 mos.	—	10*	15*	60*	0*	—
	3 mos.	—	10*	35*	60*	53*	—

* Single pot.

For all of these forms except *concolor* epicotyl treatment was carried out as for *L. auratum* with essentially the same results (Table VI). Limited epicotyl tests for *L. szovitsianum* are also reported. Roots were produced for this form at 20° C. in peat.

It is quite possible that a period longer than three months is required for the complete initiation of growth of the leaves of *japonicum* and

szovitsianum seedlings. Longer periods were not tried because no seedlings were available.

Flat plantings of the 1934 crop of *canadense*, *japonicum*, *rubellum*, and *superbum* made in December 1934 and January 1935 and placed in a mulched frame produced no seedlings in 1935 but from 62 to 78 per cent was obtained in the spring of 1936. Plantings made in the greenhouse and later transferred to a mulched frame gave the same results as for *L. auratum*.

Some flats of sample plantings of seeds made in soil in a 21° C. greenhouse from various low temperatures were used subsequently for epicotyl dormancy tests as in the case of *L. auratum*. Whereas *L. auratum* samples were transferred only to the cold frames after periods in the greenhouse, samples of *canadense*, *japonicum*, *rubellum*, and *superbum* were also transferred to a room held at 10° C.

Samples of *L. canadense* seed, which had been at various low temperatures for four months, were planted in the greenhouse on February 15, 1935. These seeds had germinated and developed good roots by October 15, 1935 when the flat was transferred to 10° C. Removal to the greenhouse again after five months at 10° C. resulted in 74 to 94 per cent shoot production in one week.

A similar planting of *L. superbum* gave similar results, but 10° C. was ineffective for both forms if preceded by only two weeks in the greenhouse. Sample plantings of all of these forms transferred to the mulched frame in January 1936 after three months in the greenhouse gave excellent seedling production in May 1936.

GROWTH AND IDENTIFICATION OF SEEDLINGS

Some seedlings of all forms reported here were grown and identified, by flowers or leaves, by Mr. O'Leary. *L. concolor* has been brought into bloom in one and one-half to two years from the time of planting the seed. *L. auratum* required three to four years to reach the flowering stage. *L. canadense*, *japonicum*, *rubellum*, *superbum*, and *szovitsianum* have not yet flowered and probably will require four or five years to reach that stage.

SUMMARY

Experiments have been performed using seeds of six lilies of the so-called two-year forms to determine the cause of delay in germination. *L. auratum*, *L. canadense*, *L. japonicum*, *L. rubellum*, *L. superbum*, and *L. szovitsianum* were included in the study.

It was found that all of these seeds required from three to six months at a high temperature (about 20° C.) for the initiation and growth of the root which is non-dormant. When the root had started to grow a period of

six weeks to three months at low temperature (1° to 10° C.) was required to initiate growth of the dormant first leaves.

If greenhouse and cold room facilities are available, this method could be put into operation as soon as the seeds are harvested and lily seedlings would be obtained above ground in less than a year.

Practically, spring or summer planting outside is the solution. Tests were made to show that seeds of *L. auratum* may be successfully planted outside any month during the year with the possible exception of August, September, and October. However, a warm period must precede a cold period before leaves will be formed. Late fall or winter plantings are not harmful but have no advantage since the seeds lie inactive until the advent of warm weather in the spring.

Tests on seeds of *L. concolor* showed prompt growth of both root and cotyledon.

LITERATURE CITED

1. BARTON, LELA V. Seedling production of tree peony. Contrib. Boyce Thompson Inst. **5**: 451–460. 1933.
2. BIGELOW, P. W. Lilies from seed for beginners. Horticulture **13**: 76. 1935.
3. CRAIG, W. N. The germination of lily seeds. Horticulture **9**: 393. 1931.
4. CUTLER, LEORA H. Starting lilies from seed indoors. Horticulture **12**: 194. 1934.
5. FOX, HELEN MORGENTHAU. Garden Cinderellas: How to grow lilies in the garden. 269 pp. The Macmillan Company, New York. 1928.
6. ——— Lilies from seed in the East. Horticulture **9**: 344. 1931.
7. FRENCH, HATTIE M. The regal lily from seed. Flower Grower **20**: 109. 1933.
8. GRIFFITHS, DAVID. A score of easily propagated lilies. U. S. Dept. Agric. Circ. 23. 35 pp. 1928.
9. GROVE, A. The seminal propagation of lilies. Gard. Chron. **87**: 86. 1930.
10. ——— Germination of seeds of *Lilium giganteum*. Gard. Chron. **88**: 251. 1930.
11. INGRAM, J. Development of lily seedlings. Roy. Hort. Soc. Lily Year-Book **1935**: 83–86.
12. PRESTON, ISABELLA. Liliums from seeds in Ontario. Gard. Chron. **76**: 320. 1924.
13. PURDY, CARL. Germination of lily seeds. Horticulture **9**: 313–314. 1931.
14. RICHARDSON, MAUDE BOYCE. Growing lilies from seed. Flower Grower **22**: 401. 1935.
15. SLATE, GEORGE L. The germination of lily seeds. Horticulture **12**: 101. 1934.
16. STERN, F. C. Propagation of lilies by seed. Roy. Hort. Soc. Lily Year-Book **1933**: 119–129.
17. STOUT, A. B. Seedling lilies: A report of progress of experimental studies with species of *Lilium*. Jour. New York Bot. Gard. **25**: 185–194. 1924.
18. SUTTLE, H. D. Notes on growing lilies from seed. Flower Grower **23**: 156. 1936.
19. TAYLOR, G. M. Lilies from seed. Roy. Hort. Soc. Lily Year-Book **1932**: 72–77.
20. WESTON, T. A. Growing lilies from seed. Horticulture **9**: 419. 1931.
21. ——— Growing lilies from seed. Horticulture **12**: 420. 1934.

TUBERIZATION OF ARTICHOKES REGULATED BY CAPPING STEM TIPS WITH BLACK CLOTH

P. W. Zimmerman and A. E. Hitchcock

The development of underground food storage organs of plants is known to be conditioned by relative length of day and night. Both storage roots and underground stems (tubers) are so involved. Photoperiodism must therefore be considered in connection with food storage as well as for the induction of flowers.

Garner and Allard (2) in 1923 reported that the growth of underground stems and the ultimate production of tubers was governed by the relative length of day and night. They showed that Jerusalem artichokes under natural light conditions first grow long underground stems and then tuberization and flowering occur as the days become shorter in September and October. If, however, the day length was artificially shortened the artichoke plants were induced to form flower buds and tubers during the summer months. Under such conditions the rhizome stage of growth was prohibited, the tubers being formed near the mother stem. There appears to be a given day length which favors rhizome development and a shorter period which regulates formation of tubers.

Zimmerman and Hitchcock (4) reported in 1929 that flowering and storage roots of certain dahlia varieties were concurrently induced with relatively short days. Under field conditions neither flower nor storage roots formed during long summer days. Also six hours of artificial light in addition to sunlight during the autumn months prevented storage root and flower formation of potted plants in the greenhouse. On the other hand, the same varieties of dahlias could be forced to flower and form storage roots during the long summer months when the relative day length was artificially shortened.

Knott (3) showed that it was not necessary to place the entire plant in the dark to bring about the short day flowering response; covering the tip with black cloth during part of the daylight period was sufficient to induce flower buds. This fact indicates that the flower regulating agents are located in the growing stem tips.

Arthur, Guthrie, and Newell (1, p. 507) found no relation between carbohydrate and nitrogen content and flowering in either long or short-day types. The percentages of carbohydrate and nitrogen in general could be changed by varying light intensity, length of day, or the nutrient supply in several ways. They found that tuber production in potatoes was favored by low temperature in combination with high light intensity and long days. High temperature favored growth of aerial stems but prevented

tuberization. What actually regulates the production of flower buds and tuberization is still an unanswered question.

The effect of day length on formation of storage roots of dahlias and tuberization of artichokes suggested the hypothesis that some localized part of the plant was responsible for regulating the growth of underground storage organs.

The present paper is concerned with recent experimental results which indicate that the stem tip rather than the entire plant regulates tuber formation and food storage of the underground parts of plants.

Experiments were conducted with pot-grown Jerusalem artichokes (*Helianthus tuberosus* L.) planted on May 15 and June 24. Altogether there were 54 pots, with an average of four shoots per pot, which were divided into three sets: control, kept in normal day length; one set placed in the dark from 4:30 P.M. to 9 A.M. to provide a short day; and one set used for capping the tips with black cloth during the time the second lot remained in the dark. The same kind of cloth was used for capping and covering the entire plant. The experiments were started when the shoots were 12 to 18 inches in height. The starting time for the first lot was June 30, and the second, July 16.

The soil was fertilized when the tubers were planted and refertilized each week (one teaspoonful of 5–8–7 fertilizer per six inch pot) after the light treatments began.

By August 27 the plants varied in height from approximately three to five and one-half feet. The tallest plants were among the controls. Both covering the entire plant and capping retarded stem elongation. Covering, however, was slightly more effective than capping.

The effectiveness of covering with the black cloth was tested by including a variety of hardy *Chrysanthemum nipponicum* Hort., which is known to be a typical short-day type for flowering. Six of these plants were placed with the artichokes on August 1 to be covered daily at 4:30 P.M. and uncovered at 9 A.M. All of the chrysanthemum plants were in flower by September 5 whereas those in normal day length were just beginning to form buds at that time (Fig. 1 A).

The effect of covering was also tested with *Kalanchoe daigremontiana*. Ten of these plants were placed with the artichoke plants on July 16 when the edges of all the leaves were heavily loaded with plantlets which are characteristically developed by this species during the summer. All new leaves which developed after the artificial short-day treatment began were entirely without plantlets on the leaves (Fig. 1 B).

The artichoke plants covered or capped as described did not form flower buds. The length of day may have been too short. Garner and Allard (2) reported that artichokes formed buds but did not flower with a ten-hour day.

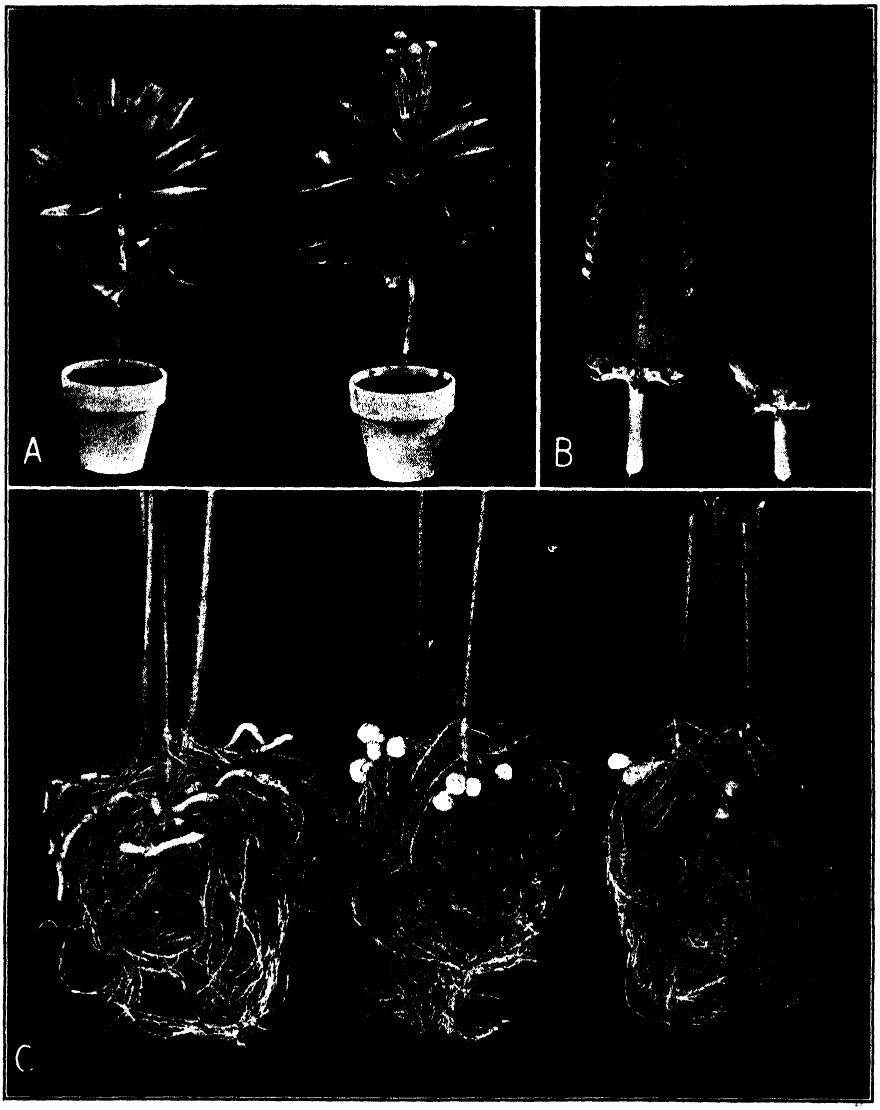


FIGURE 1. Plants showing the effects of relative lengths of day and night. A. Chrysanthemum. Left: control in normal day length; right: plant covered daily with black cloth from 4:30 p.m. to 9 a.m., August 1 to September 1. B. Kalanchoe. Left: leaf from plant in normal day length; right: leaf from plant covered daily with black cloth from 4:30 p.m. to 9 a.m., July 16 to September 1. C. Underground portions of artichokes in experiment from July 16 to August 27. Left: plant in normal day length; middle: plant covered daily with black cloth from 4:30 p.m. to 9 a.m.; right: plant with stem tips, only, capped with black cloth daily from 4:30 p.m. to 9 a.m.

On August 27 the plants were lifted from the pots and the different sets compared with reference to underground stem and tuber development. The controls in normal day length had long slender underground stems but no tubers. The covered plants had tubers but no underground stems, as reported by Garner and Allard (2). The capped sets in practically every respect responded the same as the plants completely covered. Comparative results are illustrated in Figure 1 C. Although the specimens selected for the illustration do not show the difference, the covered plants produced, as a rule, fewer and smaller tubers than the capped plants. Also, the height of the covered plants was slightly less than that of the capped ones.

The difference between the two lots which had been planted at different times was only slight. The earlier plantings produced the largest and greatest number of tubers. Also, there was some indication of tuberization in the controls at this time. The order of response was, however, the same in both lots.

As a variation from the experiments as described above, four controls of the lot planted July 16 were capped with black cloth beginning August 19. Eight days later when all plants were examined, these four differed from all other plants. They had long underground stems, most of which were beginning to tuberize at the distal ends. This response shows that capping becomes effective in a very short time, stopping rhizome development and inducing tuberization.

While the information at hand does not permit complete interpretation of the data given in the present report, there is every indication that the growing stem tip has a regulatory influence on the development of underground stems and tubers. It seems likely that the regulators are chemical agents of a hormone-like nature, manufactured in the stem tip and sent to other parts of the plant where they can exert a controlling influence on the development of the underground portions.

Whatever the controlling influence, it is evident that rhizome development and ultimate tuberization can be regulated by definite day lengths. It is possible to induce tuberization without rhizomes. If rhizomes can be induced to continue growth without tuberization then it would appear that different amounts of the same substance or two different substances operate as the controlling factors. Since no effective substances of this sort have ever been extracted from plants, the answers to such questions must await further research.

Since no flower buds were formed while tuberization was readily induced, the question also arises if different amounts of the same substance or two different substances are involved. Under field conditions in late August both rhizomes and flower buds were being developed at the same time. During the first week in September tuberization was just beginning on large plants in the field. At this time small plants of the same variety,

though started later in the season, had long underground runners but no tubers nor flower buds. The size and age of the plant may, therefore, play some part. If so, the problem looks still more complex.

The information to date indicates a correlation between rate of stem elongation and the type of development of underground parts. Medium day length slightly retards elongation; very short days greatly retard elongation. Associated with these responses are rhizome production with medium day length and tuberization with very short days. The question now arises whether factors other than light which affect rate of stem elongation might also affect rhizome production and tuberization. Experiments with synthetic growth substances which are known to retard stem elongation are now under way and may help to answer some of the questions raised.

Experiments are now being conducted to determine whether the same factors which regulate tuberization of artichokes also control the formation of storage roots of dahlias and other plants.

SUMMARY

Jerusalem artichokes were grown in pots under three sets of conditions as follows: under normal day length during summer months; plants covered with black cloth from 4:30 P.M. to 9 A.M.; stem tips, only, capped with black cloth from 4:30 P.M. to 9 A.M.

The controls in normal day length had long slender underground stems but no tubers; the covered plants had tubers but no underground stems; and the capped plants also had tubers but no underground stems, thus resembling the plants completely covered.

The results show that capping the stem tip has the same effect on underground stems and tuberization as covering the whole plant to provide a short day. The controlling influence is therefore centered in the growing tip rather than the plant as a whole.

The suggestion was made that any one of several factors which affect the rate of stem elongation might be substituted for the light factor which is known to control flowering and tuberization of plants.

LITERATURE CITED

1. ARTHUR, JOHN M., JOHN D. GUTHRIE, and JOHN M. NEWELL. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930. (*Also in* Contrib. Boyce Thompson Inst. **2**: 445–511. 1930.)
2. GARNER, W. W., and H. A. ALLARD. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agric. Res. **23**: 871–920. 1923.
3. KNOTT, J. E. Further localization of the response in plant tissue to relative length of day and night. Proc. Amer. Soc. Hort. Sci. **23**: 67–70. 1926.
4. ZIMMERMAN, P. W., and A. E. HITCHCOCK. Root formation and flowering of dahlia cuttings when subjected to different day lengths. Bot. Gaz. **87**: 1–13. 1929. (*Also in* Contrib. Boyce Thompson Inst. **1**: 467–478. 1929.)

FIELD TRIALS WITH FIBRE POTS

W. J. YOUDEN AND P. W. ZIMMERMAN

INTRODUCTION

The value of various methods of producing seedlings prior to transplanting in the field has been a subject of considerable discussion and experimentation. Clay pots have long served a very important purpose for growing plants. They are easily made, can be obtained in various shapes and sizes, are inexpensive, and can be used repeatedly. Seedlings can be removed from clay pots with only slight disturbance to the roots compared to the damage done when the plants are lifted from flats or cold frames. If fibre pots, of a sort which could be penetrated by the root system, were available, the pot and its contents could be planted in the soil with no disturbance whatever to the plant. At first thought this procedure seems entirely feasible, but several difficulties have been encountered where fibre pots were used. Some of the problems involved are: cost of producing seedlings in the various ways; the effect of the different types of pots on the growth of the seedlings; and ultimately the yield and cost of crop production. Several published reports have been concerned with some of these problems. Plants grown in paper pots seemed to be unduly retarded in growth. This result was difficult to understand until a search of the literature disclosed that cellulose-decomposing organisms utilize the nitrates of the surrounding soil, preventing plants from obtaining enough nitrogen for best growth (7). Kellerman and Wright (5) found that the addition of cellulose and straw to soil in which citrous seedlings were growing brought on a state of malnutrition. They state that green manures do not have the same effect. Viljoen and Fred (8) found that the addition of wood and wood pulp cellulose to soil had a harmful effect on plants. They attributed it to the utilization of nitrate by microörganisms which decompose the wood.

Collison and Conn (1) studied the effect of straw on plant growth. Their results agreed with the earlier work and they support the theories that the plants are forced to compete with soil organisms which use nitrates and that such organisms are greatly stimulated in growth by the presence of large supplies of energy-forming material, as substances high in carbon content.

Knott and Jeffries (6) reported the results of experiments where they compared the growth of seedlings in clay pots versus a variety of fibre pots. They found that new clay pots were not as good for growing plants as those which had been previously used because the new pots absorbed the nitrates from the soil. The bad effect could be overcome by the addition of

nitrates to the soil. Where fibre pots were used they found the amount of nitrates in the soil within a container was influenced by the presence of readily decomposable cellulose material in the wall. The addition of nitrate nitrogen to the soil was recommended to overcome the ill effect of the pot.

Haber (3) compared the value of several containers for growing plants in the greenhouse, and ranked them in the following order: clay pots, limed peat pots, flats, wood veneer bands, pulp planting pots, pulp pots, and paper bands. When, however, sodium nitrate solution was added to the soil once a week, the growth in any of the containers equalled that of the clay pots.

Jones (4) found that paper pots impregnated and bound together with asphalt produced plants equal to those grown in clay pots.

From the foregoing review of literature, it appears certain that cellulose-decomposing organisms attack the wall of fibre pots, utilizing the nitrogen of the soil and bringing about a state of malnutrition of plants growing therein; also it appears that the ill effects can be overcome by the addition of nitrate to the soil in excess of that utilized by the microörganisms.

This report deals with field trials conducted in 1932 and 1933. The purpose was to determine the relative yields of plants seeded in flats, wood cellulose pots, new clay pots, fibre pots, and fibre pots with the addition of sodium nitrate. All were seeded at the same time and kept in the greenhouse until ready for transplanting in the field. The usual planting procedure was followed with the plants started in flats. In the case of the clay pots the plants were removed with as little disturbance to the root system as possible. The fibre pots were simply buried intact. The 1932 trials included ten different fibre pots[1] made up from varying proportions of ground wood, newsprint, German moss peat, tobacco stem meal, "Fertobac," and rabbit hair. The following test plants were used: tomato (*Lycopersicon esculentum* Mill. varieties Bonny Best and Marglobe), eggplant (*Solanum melongena* L. var. *esculentum* Nees.), pepper (*Piper nigrum* L.), and cabbage (*Brassica oleracea* L. var. *capitata* L.). The results showed that plants grown in the pots gave greater yields than the plants started in flats but did not show any one pot consistently superior to the others. The number of pots was reduced to four the following year and two varieties of tomato used as test plants. The arrangement of the field trials and the interpretation of the yields obtained in the 1933 experiment will be given in detail.

ARRANGEMENT OF THE FIELD TRIALS IN 1933

The original concept of the experiment was a simple comparison of the yields obtained from plants started in a fibre pot, a clay pot, and in flats.

[1] Furnished by L. M. Burt Company, Ltd., Buffalo, New York.

To this list was added a cellulose pot, and the fibre pot soaked in one per cent sodium nitrate solution. It was desirable to have a fairly broad basis of comparison for these five items rather than rate them under one arbitrary set of conditions. This was achieved by using two different soil conditions, two sizes of pots, and two varieties of tomato as test plants. All eight combinations of these three factors were employed. The soil for seeding was taken directly from the field to which the plants were ultimately transferred. One-half of the containers were filled with the soil as obtained from the field, one-half with the same soil after addition of complete fertilizer (4–8–7) in the proportion of 1:100. One-third of the flats were thinned to 80 plants per flat and the remainder to 40 plants per

TABLE I
CODE NUMBERS ASSIGNED TO TREATMENTS

	Field soil				Field soil+1:100 fertilizer			
	Three-inch*		Four-inch*		Three-inch		Four-inch	
	B.*	M.*	B.	M.	B.	M.	B.	M.
Flat	11	12	13	14	15	16	17	18
Paper	21	22	23	24	25	26	27	28
Clay	31	32	33	34	35	36	37	38
Fibre	41	42	43	44	45	46	47	48
Fibre+NO_3	51	52	53	54	55	56	57	58

* In the case of flats, three-inch corresponds to 80 plants per flat and four-inch corresponds to 40 plants per flat. B and M refer to Bonny Best and Marglobe varieties.

flat, to give a division corresponding to the three and four-inch sizes in the pots. For convenience in terminology the headings three-inch and four-inch will be used to designate these flats as well as the pot sizes. The eight combinations of the three factors, soil, pot size, and variety of test plant, coupled with the five items under test gave a total of 40 different treatments. Table I shows the various treatments and the code number assigned to each. The first figure of the code number denotes the type of pot and the second the soil, pot size, and variety. For example, all the Marglobe plants have even numbers.

The arrangement of the treatments in the field presented more of a problem than in 1932. In that year there were 12 items under test and these were arranged at random in a compact block of 12 rows. The block was then replicated six times. The variation in yields of these blocks indicated that it was useless to attempt to make the blocks large enough to include all 40 treatments. Accordingly a scheme was adopted of dividing the available ground into 25 rectangles 32 by 48 feet to which the pots were assigned according to the Latin Square arrangement described by Fisher (2). Within each of the 25 rectangles there were eight 12-hill rows

Row No.	Soil	Pot Size	Variety	Ripe	Green	Total
21		3	B	75.3	12.3	87.6
26	+	3	M	98.5	4.5	103.0
25	+	3	B	163.6	4.2	167.8
24		4	M	104.6	4.9	109.5
27	+	4	B	121.5	3.8	125.3
28	+	4	M	124.9	4.4	129.3
23		4	B	56.4	3.2	59.6
22		3	M	53.6	4.0	57.6
47	+	4	B	153.3	2.0	155.3
46	+	3	M	148.5	3.8	152.3
42		3	M	94.9	2.6	97.5
45	+	3	B	92.8	3.9	96.7
44		4	M	78.8	1.6	80.4
43		4	B	110.4	1.0	111.4
41		3	B	93.7	.4	94.1
48	+	4	M	167.2	2.5	169.7
34		4	M	150.5	4.1	154.6
37	+	4	B	98.2	1.4	99.6
32		3	M	150.9	6.0	156.9
38	+	4	M	211.0	10.3	221.3
35	+	3	B	155.0	4.4	159.4
33		4	B	137.8	3.7	141.5
36	+	3	M	155.0	7.7	162.7
31		3	B	110.6	2.6	113.2
56	+	3	M	178.5	12.5	191.0
52		3	M	184.0	11.2	195.2
58	+	4	M	172.7	9.6	182.3
54		4	M	145.9	6.7	152.6
57	+	4	B	110.2	.9	111.1
55	+	3	B	96.9	2.3	99.2
53		4	B	93.0	4.2	97.2
51		3	B	80.6	1.5	82.1
12		3	M	99.7	5.8	105.5
11		3	B	85.9	1.3	87.2
14		4	M	99.3	5.4	104.7
15	+	3	B	100.5	.4	100.9
13		4	B	127.8	1.0	128.8
17	+	4	B	115.5	1.1	116.6
18	+	4	M	121.6	6.1	127.7
16	+	3	M	119.8	4.1	123.9

Row No.	Soil	Pot Size	Variety	Ripe	Green	Total
53		4	B	163.4	1.2	164.6
57	+	4	B	183.5	.5	184.0
55	+	3	B	162.4	1.0	163.4
56	+	3	M	148.1	2.2	150.3
58	+	4	M	173.5	.9	174.4
54		4	M	196.9	1.6	198.5
52		3	M	205.1	3.2	208.3
51		3	B	169.1	1.2	170.3
21		3	B	68.3	.3	68.6
[illegible]	+	3	M	129.6	1.0	130.6
24		4	M	58.9	4.3	63.2
28	+	4	M	93.7	2.6	96.3
25	+	3	B	55.3	.0	55.3
23		4	B	31.1	.0	31.1
22		3	M	33.0	1.0	34.0
27	+	4	B	20.1	.0	20.1
12		3	M	44.4	.4	44.8
13		4	B	28.2	.0	28.2
11		3	B	35.8	5	36.3
17	+	4	B	90.5	.5	91.0
15	+	3	B	128.7	4.5	133.2
16	+	3	M	173.4	7.9	181.3
14		4	M	155.2	5.2	160.4
18	+	4	M	113.4	4.4	117.8
48	+	4	M	127.3	2.8	130.1
45	+	3	B	95.6	.2	95.8
44		4	M	107.0	4.8	111.8
46	+	3	M	124.4	4.8	129.2
42		3	M	90.6	6.7	97.3
47	+	4	B	127.9	.0	127.9
41		3	B	80.3	1.5	81.8
43		4	B	100.6	.0	100.6
33		4	B	134.0	1.2	135.2
31		3	B	113.2	1.0	114.2
35	+	3	B	110.5	.5	111.0
37	+	4	B	129.9	.0	129.9
32		3	M	168.0	4.2	172.2
36	+	3	M	141.5	4.6	146.1
38	+	4	M	139.0	2.0	141.0
34		4	M	184.4	2.1	186.5

Row No.	Soil	Pot Size	Variety	Ripe	Green	Total
38	+	4	M	193.0	3.7	196.7
35	+	3	B	219.0	1.4	220.4
33		4	B	177.5	.3	177.8
37	+	4	B	198.3	.4	198.7
31		3	B	132.0	2.4	134.4
34		4	M	160.6	7.5	168.1
32		3	M	170.1	6.7	176.8
36	+	3	M	155.2	3.5	158.7
52		3	M	141.0	1.7	142.7
57	+	4	B	138.6	.7	139.3
58	+	4	M	189.2	3.7	192.9
51		3	B	127.6	.8	128.4
54		4	M	166.8	2.7	169.5
56	+	3	M	158.6	1.1	159.7
55	+	3	B	121.6	.3	121.9
53		4	B	127.7	.5	128.2
42		3	M	125.7	2.8	128.5
41		3	B	54.8	.4	55.2
46	+	3	M	144.6	3.3	147.9
45	+	3	B	131.2	1.5	132.7
43		4	B	102.7	1.2	103.9
48	+	4	M	223.7	8.3	232.0
44		4	M	144.4	6.4	150.8
47	+	4	B	128.2	1.6	129.8
13		4	B	78.4	1.8	80.2
17	+	4	B	69.7	1.0	70.7
12		3	M	88.9	2.8	91.7
16	+	3	M	89.6	3.3	92.9
14		4	M	75.9	5.0	80.9
15	+	3	B	42.0	.2	42.2
11		3	B	60.5	1.2	61.7
18	+	4	M	142.0	9.3	151.3
24		4	M	119.9	8.5	128.4
22		3	M	119.5	6.8	126.4
27	+	4	B	158.5	1.3	159.8
25	+	3	B	148.8	4	149.2
23		4	B	123.1	1.1	124.2
28	+	4	M	184.1	6.3	190.4
21		3	B	79.5	1.1	80.6
26	+	3	M	152.8	6.9	159.7

Row No.	Soil	Pot Size	Variety	Ripe	Green	Total
45	+	3	B	196.1	5.3	201.4
44		4	M	123.4	10.0	133.4
42		3	M	157.0	9.6	166.6
46	+	3	M	220.2	9.8	230.0
41		3	B	129.9	1.0	130.9
47	+	4	B	164.4	1.5	165.9
48	+	4	M	211.5	5.5	217.0
43		4	B	102.7	1.1	103.8
13		4	B	146.6	1.1	147.7
11		3	B	133.5	.4	133.9
16	+	3	M	143.4	4.1	147.5
17	+	4	B	112.8	1.3	114.1
12		3	M	122.0	3.2	125.2
18	+	4	M	164.6	2.9	167.5
14		4	M	160.0	3.0	163.0
15	+	3	B	142.0	.2	142.2
22		3	M	85.2	2.7	87.9
27	+	4	B	114.7	.2	114.9
25	+	3	B	137.5	1.1	138.6
21		3	B	91.7	2.0	93.7
26	+	3	M	162.1	7.1	169.2
23		4	B	96.4	1.1	97.5
24		4	M	126.7	7.4	134.1
28	+	4	M	134.5	3.5	138.0
38	+	4	M	126.3	3.7	130.0
34		4	M	117.6	2.0	119.6
31		3	B	82.4	2.5	84.9
32		3	M	129.8	3.9	133.7
36	+	3	M	176.7	5.4	182.1
37	+	4	B	84.0	.7	84.7
35	+	3	B	77.1	1.8	78.9
33		4	B	115.2	1.3	116.5
56	+	3	M	152.0	5.3	157.3
55	+	3	B	108.2	6	108.8
53		4	B	133.6	.3	133.9
57	+	4	B	117.7	1.4	119.1
58	+	4	M	214.5	1.7	216.2
54		4	M	245.4	4.0	249.4
51		3	B	136.8	.4	137.2
52		3	M	172.9	5.0	177.9

Row No.	Soil	Pot Size	Variety	Ripe	Green	Total
18	+	4	M	172.4	9.4	181.8
17	+	4	B	190.3	3.7	194.0
11		3	B	164.8	2.9	167.7
15	+	3	B	180.0	5.0	185.0
16	+	3	M	203.9	8.1	212.0
13		4	B	153.8	5.4	159.2
12		3	M	139.0	4.7	143.7
14		4	M	123.2	5.9	129.1
35	+	3	B	136.4	7.1	143.5
38	+	4	M	163.6	3.3	166.9
36	+	3	M	171.2	3.2	174.4
34		4	M	148.7	6.8	155.5
37	+	4	B	155.8	1.3	157.1
32		3	M	165.7	3.3	169.0
33		4	B	140.9	1.1	142.0
31		3	B	128.6	1.0	129.6
55	+	3	B	114.3	.0	114.3
52		3	M	153.9	2.6	156.5
57	+	4	B	168.0	2.5	170.5
54		4	M	172.1	5.7	177.8
53		4	B	184.3	3.0	187.3
51		3	B	178.4	2.4	180.8
56	+	3	M	197.5	10.8	208.3
58	+	4	M	199.9	8.1	208.0
24		4	M	97.8	6.1	103.9
21		3	B	76.3	.7	77.0
27	+	4	B	164.8	4.2	169.0
22		3	M	81.8	5.6	87.4
26	+	3	M	168.1	8.6	176.7
23		4	B	85.7	1.3	87.0
25	+	3	B	102.0	1.4	103.4
28	+	4	M	149.5	5.7	155.2
44		4	M	115.4	10.5	125.9
46	+	3	M	184.9	5.8	190.7
47	+	4	B	161.7	.5	162.2
48	+	4	M	205.3	4.0	209.3
41		3	B	118.3	1.0	119.3
43		4	B	99.3	1.0	100.3
45	+	3	B	110.2	.0	110.2
42		3	M	104.4	6.0	110.4

FIGURE 1. Scale drawing (one inch equals 37 feet) of field showing arrangement of rows and yields. Symbols: +, fertilized soil; B, Bonny Best; M, Marglobe.

which were assigned at random to the eight combinations of soil, pot size, and variety. The unit in harvesting was a row of 12 hills. Each plot of the Latin Square contained 96 hills, all of the same pot. The reduction in the size of the replicated block by this procedure added materially to the accuracy of the trials. Another result of this arrangement is that some of the comparisons among the treatments may be made more accurately than others. It is apparent that comparisons within a rectangle will have a higher precision than those involving different rectangles. This is not necessarily a disadvantage since the contrasts of greatest interest or of most difficulty of detection may be assigned to the favorable locations. Figure 1 shows a scale drawing of the field and the treatment and yield of each of the 200 rows.

SEEDLING CULTURE

All the pots and flats were seeded on April 11. About four seeds were placed in each pot and on April 25 all seedlings but one were removed. In another week it was apparent that the plants in the unfertilized field soil were not growing satisfactorily and on May 3 these were watered with a nutrient solution. This was repeated five times at intervals of two or three days. At each watering there were used 30 liters of solution containing 25 grams each of potassium nitrate, magnesium sulphate, and potassium dihydrogen phosphate, 50 grams of calcium nitrate, and a few drops of one per cent ferric chloride solution. The plants in the nitrated pots with field soil received the nutrient solution although they did not appear to need it. The plants in the paper pots did very poorly until watered with the nutrient solution.

It was unfortunate that the fertility of the field soil was so low as to require the addition of this nutrient. One interpretation of the increased yield of plants grown in pots over those started in flats was based on the contention that the pots carried into the field a larger portion of the fertile soil than the flat seedlings. By using the field soil as a starting medium it had been hoped to gain information regarding this point.

The appearance of the plants on May 15 just prior to their transfer to the field is shown in Figure 2. Section A shows the progress of Marglobe plants in the unfertilized field soil. The group brings out the differences between the several pots and indicates the relatively small effect of pot diameter. The only satisfactory plants were the two in the nitrate fibre pots. The seedlings in the clay pots were next best, with those in the plain fibre and cellulose pots much poorer than the flat seedling shown in the center of the group. Figure 2 B shows the same contrasts when fertilized soil was used. The effect of pot size was more noticeable since the four-inch pots held about twice as much soil as the three-inch pots. Thus the plants in the four-inch clay, cellulose, and plain fibre pots were better than those in the corresponding three-inch pots. There was no appreciable difference

between the plants in the three and four-inch nitrate fibre pots. Ccr-responding pots (e.g., 22 and 26) in the two sections may be compared to show the soil contrast. As would be expected the use of fertilized soil in place of field soil had a marked effect in all cases save the nitrate fibre pot which already carried an adequate supply of nitrogen. It will be of interest subsequently to correlate the appearance of these plants with the yield.

On May 15 the hardening off process was started and on the 19th and 20th the plants were transferred to the field. In the case of the flats as

FIGURE 2. Appearance of Marglobe plants just before transfer to the field. A. Plants in unfertilized soil. B. Plants in fertilized soil.

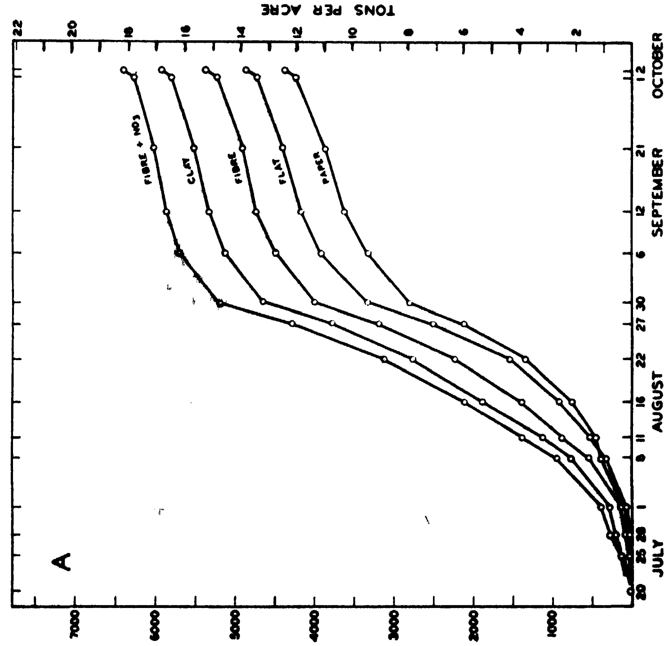
much soil as the spacing would permit was kept intact about the roots. It was necessary, however, to water immediately after transplanting to prevent wilting. The paper, fibre, and the nitrate fibre pots were left on and buried in the field. The roots of the plants were evident on the outside of the pots. The clay pots were carefully removed and the soil about the roots left intact. None of the plants grown in pots wilted after transfer to the field. The plants were spaced four feet apart in rows which were also four feet apart. On May 12 complete fertilizer (4–8–7) at the rate of 1500 pounds per acre was added to the field and on June 26 when plants began to set fruit 200 pounds of ammonium sulphate were added to the experi-

mental plot (38,400 square feet). Surplus plants were planted nearby and drawn upon during the next few days to insure a complete stand at the beginning of the field experiment.

RESULTS OBTAINED IN THE FIELD

The entire field was harvested 15 times on the dates shown on the base line of the yield curves given in Figures 3 and 4. During the season 3000 weights were obtained and it was possible to make a comparison of all the treatments at any given harvest date. The data may be advantageously presented in the form of yield curves showing the cumulative yield to any given date. Forty of these curves could be constructed but the important conclusions are represented by the curves in Figures 3 and 4 which contrast the performances of the several pots, the soils, pot sizes, and the varieties. These curves illustrate an important feature of complex experiments. The 40 different treatments arise from four factors, namely, type of pot, pot size, soil, and variety of tomato. Any one yield may be considered as embodying the result of some combination of these four factors. Thus 20 of the yields are from Marglobe plants and an exactly corresponding 20 are from Bonny Best plants. The 20 pairs of yields may be examined for varietal difference by applying Student's method if desired. Similarly the same 40 yields may be divided into two sets of 20, contrasting the four-inch pots with the three-inch pots, and also into two corresponding sets of 20 that bring out the effect of fertilizing the soil. Finally the 40 yields may be regrouped into sets of eight on the basis of the pots. The eight values belonging to any pot may be matched with the eight from any other pot forming eight pairs which may be evaluated by Student's method. It will be noted that not only do the data serve to establish the relative yields of the pots, but that the entire experiment is used to compare in turn the pot sizes, the soils, and the varieties. Furthermore, each of these contrasts rests on a diversified base. The varieties, for example, have been compared in every possible combination of two soils, five pots, and two pot sizes. It may be of considerable interest to examine the relative performance under some particular combination of circumstances but it is no less important to establish relationships which are not limited to some unique set of conditions. The curves in Figure 3 use all the data. The curves in Figure 4 B are based on part of the data since in this case specific situations are of interest. Even here, however, the pot sizes and soils have been grouped together thus making 240 plants available for establishing each curve.

The performance curves shown in Figure 3 A for the five pots may be considered as representing the average showing when used in two pot sizes, two soils, and two varieties of tomatoes. The order of performance is in accord with expectation based on past experience, especially in the



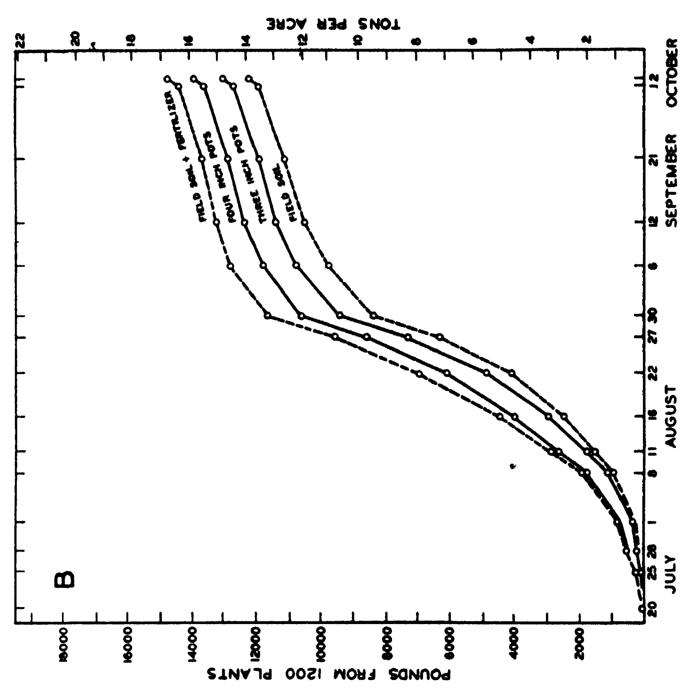


FIGURE 3. A. Cumulative yields of tomatoes grown in different pots. Each curve is based upon a group of 480 plants. Each group of 480 is the sum of eight sets of 60 plants, the eight sets differing in variety, pot size, and soil. B. The same 2400 plants used for the curves in A have been grouped on the basis of pot size and also on the basis of the soil used in the pots.

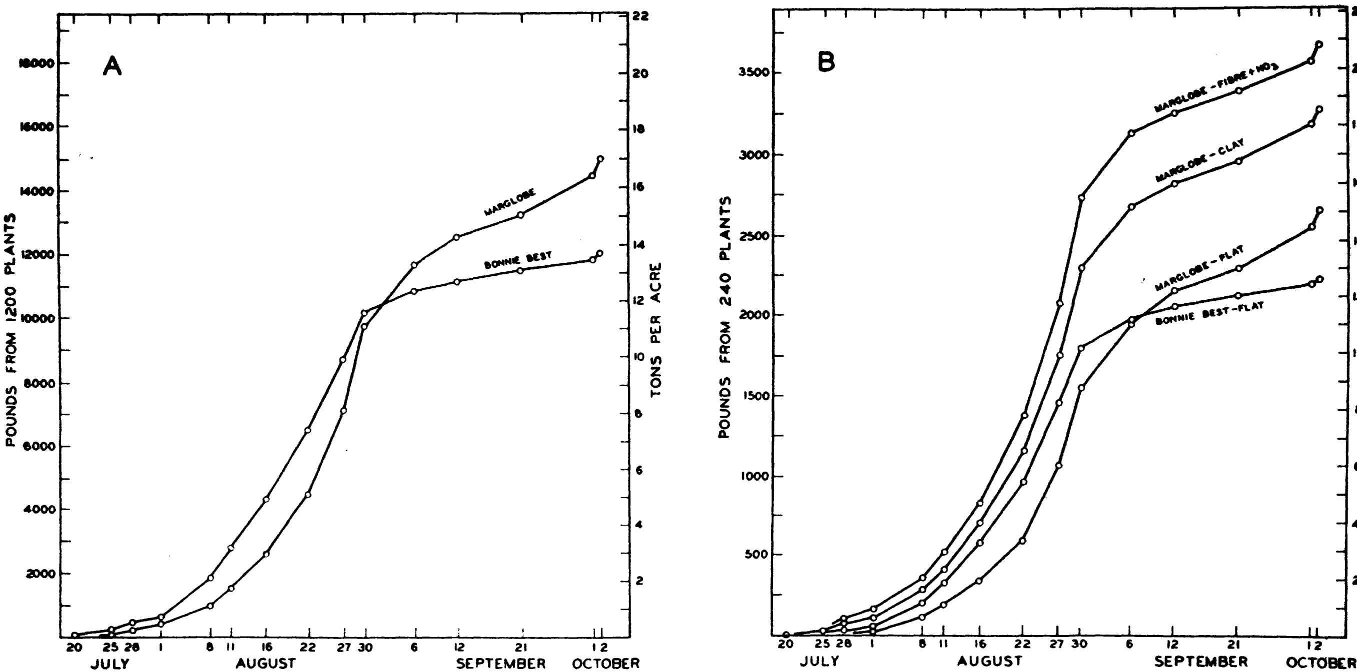


FIGURE 4. A. Comparison of Marglobe and Bonny Best tomatoes. The 1200 Bonny Best plants include 20 sets of 60 plants that differ in pot, pot size, and soil. The same holds for the Marglobe curve. B. These curves show the yields of Marglobe and Bonny Best in flats and the improvement resulting from the use of pots. Four sets of 60 plants grown in different pot sizes and soils comprise the total of 240 plants for each curve.

matter of the poor yields with the paper pots. Of particular interest is the pronounced effect of the small amount of sodium nitrate when added to the fibre pot. The average amount taken up by the small pots was 0.71 gram and by the large pots 1.46 grams. The average of these two amounts multiplied by 2720 (the number of plants per acre) gives about 6.5 pounds of sodium nitrate as being responsible for a final difference in yield of 5800 pounds per acre.

The yield curves shown in Figure 4 A for the two varieties of tomatoes are in keeping with the known early yielding characteristic of Bonny Best and the sustained yielding quality of the Marglobe variety. Figure 4 B shows that the use of pots with Marglobe made this variety earlier than the Bonny Best grown in flats. Finally, the curves in Figure 3 B show, as would be anticipated, the importance of a fertile starting soil but also show a relatively small gain due to the use of a four-inch pot in place of a three-inch pot. The relationships brought out by the yield curves verify the previously mentioned opinions based on the photographs of the plants.

The data are represented in greater detail in Figure 5. The heights of the 40 columns show the relative yields of all the treatments for the whole season. The columns have also been divided to depict the yields for the first five pickings and for the first ten pickings. The groups of eight columns sharply emphasize the pot differences while the columns within any group show to what extent the yields with any given pot are influenced by other conditions. The paper and plain fibre pots are evidently particularly susceptible to variation in the soil character.

Reference to the code numbers in Table I shows that any odd code number represents a Bonny Best yield and the immediately following even number a Marglobe yield under parallel conditions. In 19 of 20 possible cases the even numbered columns exceed in total height the corresponding odd numbers. At the end of the first five pickings the yields (shown by the lower of the two lines dividing each column) of the Bonny Best invariably exceed those of the Marglobe. The upper of the two dividing lines shows that at the end of the tenth picking Bonny Best outyielded Marglobe in 13 out of the 20 conditions, the most favorable growing conditions being the ones in which Marglobe first overtook the Bonny Best. It is instructive to note that in Figure 4 A the yield curves for the two varieties under all conditions cross between the tenth and eleventh pickings.

STATISTICAL ANALYSIS OF THE DATA

The total yield figures given in complete detail in Figure 1 have been used to construct Tables II, III, and IV. Table II brings out the Latin Square arrangement of the plots and by means of the column and row totals demonstrates the wide range of fertility exhibited by the strips into which the field was divided. The entries in Table II together with the

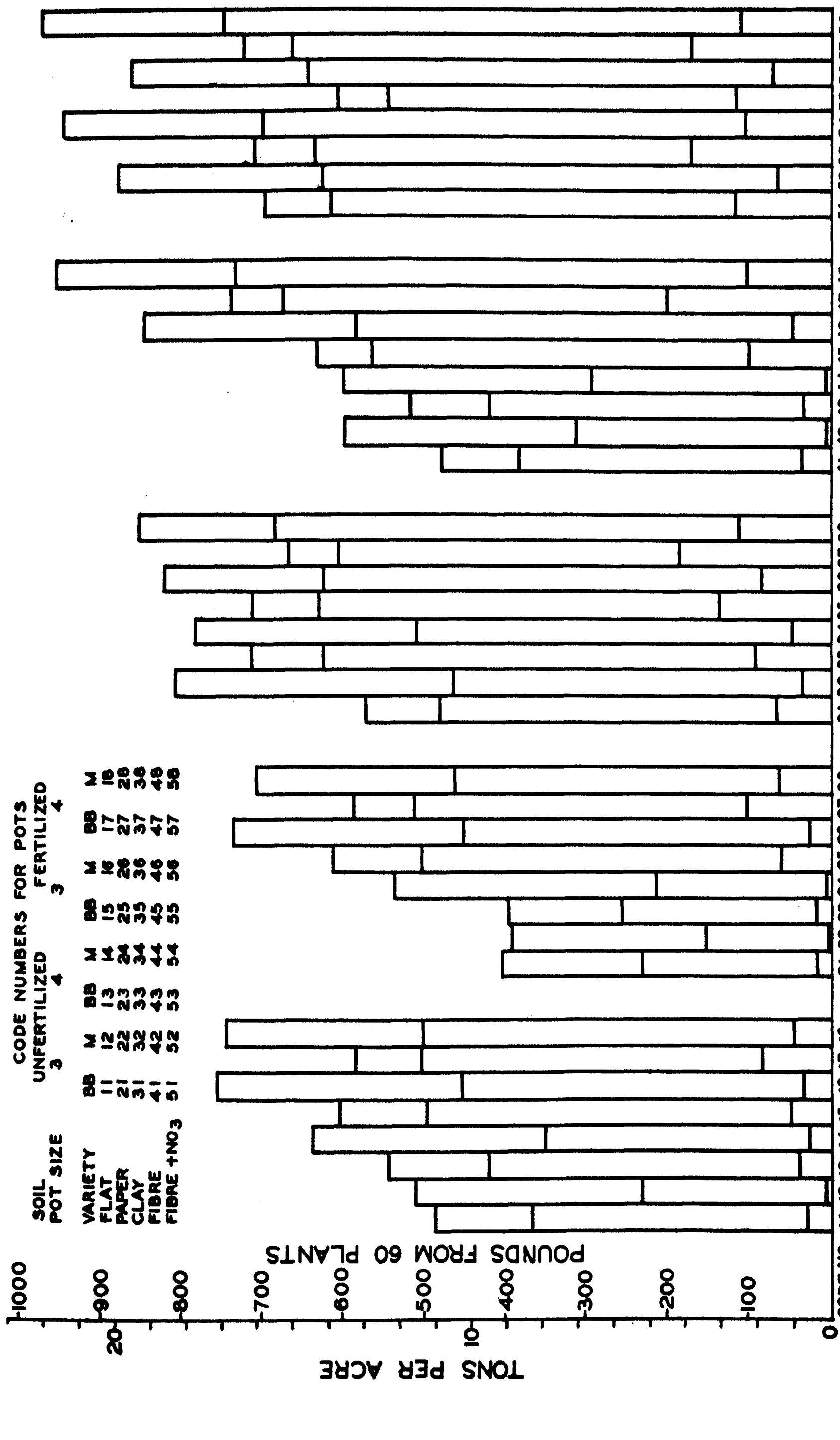


FIGURE 5. Diagram showing the relative yields of the 40 treatments at the end of the fifth harvesting, the tenth harvesting, and at the end of the season.

total yields for the different pots (Table III) are used for the analysis of variance given below.

TABLE II

TOTAL YIELD IN POUNDS FROM EACH BLOCK OF EIGHT ROWS. DATA FROM FIGURE 1

Paper 839.7	Fibre 957.4	Clay 1209.2	Flat 895.3	Fibre+NO_3 1110.7	Row totals 5012.3
Fibre+NO_3 1413.8	Paper 499.2	Flat 793.0	Clay 1136.1	Fibre 874.5	4716.6
Clay 1431.6	Fibre+NO_3 1182.6	Fibre 1080.8	Paper 1118.7	Flat 671.6	5485.3
Fibre 1349.0	Flat 1141.1	Paper 973.9	Fibre+NO_3 1299.8	Clay 930.4	5694.2
Flat 1372.5	Clay 1238.0	Fibre+NO_3 1403.5	Fibre 1128.3	Paper 959.6	6101.9
6406.6*	5018.3	5460.4	5578.2	4546.8	27010.3

* Column totals.

TABLE III

EFFECT OF SOIL ON YIELDS FROM DIFFERENT POTS. DATA FROM FIGURE 1

Pot	Field soil		Fertilized soil	
	Lbs. from 240 hills	Tons per acre	Lbs. from 240 hills	Tons per acre
Flat	2179.9	12.36	2693.6	15.28
Paper	1739.3	9.87	2651.8	15.04
Clay	2882.2	16.35	3063.1	17.37
Fibre	2203.9	12.50	3186.1	18.07
Fibre+NO_3	3238.4	18.37	3172.0	17.99

TABLE IV

TOTAL YIELDS BASED ON VARIETY, POT SIZE, AND SOIL. DATA FROM FIGURE 1

Soil	Pot size, inches	Variety	Pounds of tomatoes from 300 hills	Tons per acre
Field	3	Bonny Best	2650.7	12.03
	3	Marglobe	3193.7	14.49
	4	Bonny Best	2887.7	13.10
	4	Marglobe	3511.6	15.93
Field+4-8-7 fertilizer at the rate of 1:100	3	Bonny Best	3175.4	14.41
	3	Marglobe	4037.5	18.32
	4	Bonny Best	3310.6	15.02
	4	Marglobe	4243.1	19.25
Standard deviation			130.3	0.59

Analysis of Variance for Latin Square

Variance due to:	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Mean Square Single Row Basis*
Horizontal strips	4	240,763.79		
Vertical strips	4	384,454.17		
Pots	4	522,684.10	130,671.03	16,333.88
Error	12	245,598.69	20,466.56	2,558.32
	24	1,393,500.75		

Standard deviation single block = 143.06 pounds.
Standard deviation total of five blocks = $143.06\sqrt{5} = 319.9$ pounds.

The mean squares for pots and error differ significantly with odds of better than 100:1.

The comparison of soils, pot sizes, and varieties is made by matching corresponding rows within each of the 25 rectangles. The analysis of variance for this situation is shown below:

Variance due to:	*D. F.*	*Sum of Squares*	*Mean Square*
Blocks	24	174,187.59	
Treatments	7	82,003.17	11,714.74
Treatments and pots	28	33,723.53	1,204.41
Error	140	95,824.45	684.46
	199	385,738.74	

Standard deviation single row = 26.06 pounds.
The sum of squares opposite treatments is composed of the following parts:

Variance due to:	*D. F.*	*Mean Square*
Variety	1	43,852.42
Pot size	1	4,011.40
Fertilizer	1	31,825.12
Variety and fertilizer	1	1,970.03
Variety and pot size	1	114.44
Pot size and fertilizer	1	229.19
Pot size, variety, and fertilizer	1	.57
	7	82,003.17

The seven values for the mean square when compared with the value 684.46 obtained for error show that variety and soil were the most influential factors in determining the yield of tomatoes. The difference in yield between the three and four-inch pots, amounting to about 6 per cent, is much less important, the odds being between 20 and 100 to 1 in this case. There is some indication that the two varieties responded differently to the addition of fertilizer to the starting soil but the odds are not significant. The other interactions are of no consequence.

The entry 33,723.53 for the interaction of pots and treatments is accounted for to the extent of 20,634.75 by the differential response of the

pots with the different soils. The mean square for this interaction is 5,158.69 and therefore highly significant. Table III shows the influence of the soil on the yields from the different pots. The interactions of pots with variety and pots with pot size are not significant, that is, the relative rating of the pots was not influenced by variety or pot size. The interactions involving three or more factors are of no consequence.

The precision of the experiment is indicated in the example of the comparison of pot sizes. There were 100 rows of three-inch pots and 100 rows of four-inch pots. The standard deviation of a single row is $\sqrt{684.46}$ or 26.06. The standard deviation of the mean of 100 rows is 2.606 or just under 2 per cent of the mean row. The standard deviation of a single block of eight rows all with the same pot is 143.06 and the standard deviation of the mean of five blocks is 64.0 or 6 per cent of the mean block. Since there were but 40 rows of any given pot, the pots cannot be compared as precisely as the factors for which 100 rows were available.

SUMMARY

Field trials were made under eight different starting conditions of wood cellulose pots, flats, fibre pots, clay pots, and fibre pots soaked in 1 per cent sodium nitrate solution as containers for starting plants in the greenhouse. The containers are listed in order of increasing yield using tomato as a test plant. The yields confirm previous reports of the detrimental action of paper pots. This ill effect may be overcome by fertilizing the starting soil. The same situation holds in respect to the fibre pots. The fibre pots are also greatly improved by impregnating the pot wall with a small amount of sodium nitrate. For example, the nitrate-treated pots produced plants which yielded nearly one-third more than the flat plants. The increment in yield due to the use of nitrate in average amount just over one gram per pot amounted to 890 pounds of tomatoes per pound of sodium nitrate. The plants in pots were approximately ten days earlier than flat plants in the production of tomatoes.

The eight different starting conditions included a comparison of three and four-inch pots, different starting soils, and two varieties of tomatoes. The yields from the plants in the smaller pots were but 6 per cent under those grown in the four-inch pots. Yield curves show Bonny Best to be earlier than Marglobe, the final yield, however, being about 80 per cent of that obtained from the Marglobe variety. The use of pots with Marglobe plants not only increased the yield but gave earlier yields than Bonny Best started in flats.

In all, 40 different combinations of pots, pot sizes, soils, and varieties were tested. The use of the split block Latin Square arrangement made it possible to test this large number of treatments using comparatively small numbers of plants on a field showing wide differences in fertility in different portions of the test area.

LITERATURE CITED

1. COLLISON, R. C., and H. J. CONN. The effect of straw on plant growth. New York [Geneva] Agric. Exp. Sta. Tech. Bull. 114. 35 pp. 1925.
2. FISHER, R. A. Statistical methods for research workers. 6th ed. 339 pp. Oliver & Boyd, Edinburgh. 1936.
3. HABER, E. S. The effect of various containers on the growth of vegetable plants. Iowa Agric. Exp. Sta. Bull. 279: 151–164. 1931.
4. JONES, LINUS H. Flower pot composition and its effect on plant growth. Massachusetts Agric. Exp. Sta. Bull. 277: 148–161. 1931.
5. KELLERMAN, KARL F., and R. C. WRIGHT. Relation of bacterial transformations of soil nitrogen to nutrition of citrous plants. Jour. Agric. Res. **2**: 101–113. 1914.
6. KNOTT, J. E., and C. D. JEFFRIES. Containers for plant growing. Pennsylvania Agric. Exp. Sta. Bull. 244. 16 pp. 1929.
7. KRÜGER, W., und W. SCHNEIDEWIND. Zersetzungen und Umsetzungen von Stickstoffverbindungen im Boden durch niedere Organismen und ihr Einfluss auf das Wachstum der Pflanzen. Landw. Jahrb. **30**: 633–648. 1901.
8. VILJOEN, J. A., and E. B. FRED. The effect of different kinds of wood and of wood pulp cellulose on plant growth. Soil Sci. **17**: 199–212. 1924.

POSSIBLE RELATIONSHIP OF STANLEY'S CRYSTALLINE TOBACCO-MOSAIC-VIRUS MATERIAL TO INTRA-CELLULAR INCLUSIONS PRESENT IN VIRUS INFECTED CELLS[1,2]

HELEN PURDY BEALE

Two types of cell inclusions are characteristically present in the cytoplasm of Turkish tobacco cells affected with certain strains of tobacco mosaic virus, viz., the vacuolate, plasma-like inclusions, first referred to as "x-bodies" by Goldstein (Bull. Torrey Bot. Club **51**: 261–273. 1924), characteristic of a number of plant and animal virus diseases, and the crystalline material, often striated, which Iwanowski first described as tending to form needle-shaped crystals under the influence of acid (Zeitschr. Pflanzenkrankh. **13**: 1–41. 1903). The crystalline material occurs in the form of plates, definitely hexagonal at times, in side view oblong.

A piece of epidermis was stripped from the back of the midrib of a Turkish tobacco leaf (*Nicotiana tabacum* L. var. Turkish) affected with Johnson's tobacco virus 1 or 6. The tissue was mounted in water on a slide and brought into focus under high magnification, about 1000 (2 mm. oil immersion). HCl of approximately pH 1.3 was run under the cover slip and as the acid penetrated the cells, the oblong crystalline masses developed marked cross striations, finally breaking up into needle-shaped crystals that floated out free in the cells. The crystalline plates disintegrated into honeycombed, granular-appearing masses, which were the ends of the needle-shaped crystals with their long axes at right angles to the basal plane of the plate. In gross appearance the needle crystals were indistinguishable from the needle crystals formed upon acidification of virus extract, purified according to the method of Stanley (Science **81**: 644–645. 1935). If excess acid was added after the needle crystals had formed they went into solution leaving the cell nucleus and x-bodies intact.

Intracellular precipitation of needle-shaped crystals was obtained with six different hosts of the viruses. Three different acids were used successfully for the acid precipitation of the needle crystals.

Healthy Turkish tobacco plants and those affected with the tobacco ring-spot virus or with potato x virus gave negative results when treated with acid.

The striking similarity in gross appearance of the needle crystals obtained in the cells and by Stanley's method together with their similar behavior upon acidification suggests a common source in the plate crystals.

[1] Abstract of paper to be presented at the meetings of the American Phytopathological Society, Atlantic City, New Jersey, December 28–31, 1936.

[2] This article was preprinted November 24, 1936 from Contributions from Boyce Thompson Institute, Vol. 8, No. 4, 1936.

INACTIVATION OF TOBACCO MOSAIC VIRUS BY ASCORBIC ACID[1,2]

MARY LOJKIN

The reduced form of ascorbic acid in concentrations as low as 0.03 mg. per cc. can produce complete inactivation of purified preparations of the virus of tobacco mosaic. Inactivation takes place only when the ascorbic acid in the virus solution undergoes oxidation by atmospheric oxygen. Conditions which prevent the auto-oxidation of ascorbic acid or decrease its rate prevent inactivation or decrease the rate of inactivation of the virus, while the addition of copper which catalyses the auto-oxidation stimulates the inactivation. The virus remains active when ascorbic acid is oxidized in the absence of atmospheric oxygen by such oxidizing agents as iodine, 2, 6 dichlorophenolindophenol, and potassium permanganate.

Dehydroascorbic acid does not produce inactivation of the virus under conditions resulting in inactivation by the reduced form. The virus in the whole juice of tobacco plants is less readily inactivated than the purified preparation.

Tomatoes from healthy and diseased plants grown under the same conditions are equal in vitamin C content.

[1] Abstract of paper to be presented at the meetings of the American Phytopathological Society, Atlantic City, New Jersey, December 28–31, 1936.

[2] This article was preprinted November 24, 1936 from Contributions from Boyce Thompson Institute Vol. 8, No. 4, 1936.

COMPARATIVE EFFECTIVENESS OF ACIDS, ESTERS, AND SALTS AS GROWTH SUBSTANCES AND METHODS OF EVALUATING THEM[1]

P. W. ZIMMERMAN AND A. E. HITCHCOCK

During the past few years 32 growth promoting substances have been reported from this laboratory. Among them were 4 unsaturated carbon-containing gases (ethylene, acetylene, propylene, and carbon monoxide), 14 acids, 11 esters, and 1 nitrile. To this list may now be added 21 salts which induce hormone-like responses in plants. They are as follows:

Potassium α-naphthaleneacetate	Barium α-naphthaleneacetate
Potassium β-indoleacetate	Strontium α-naphthaleneacetate
Potassium β-indolepropionate	Ammonium α-naphthaleneacetate
Potassium γ-indolebutyrate	Ammonium β-indoleacetate
Potassium phenylacetate	Ammonium γ-indolebutyrate
Sodium β-indoleacetate	Trimethyl ammonium α-naphthaleneacetate
Sodium β-indolepropionate	Trimethyl ammonium β-indoleacetate
Sodium γ-indolebutyrate	Trimethyl ammonium γ-indolebutyrate
Sodium α-naphthaleneacetate	Tetramethyl ammonium α-naphthaleneacetate
Calcium α-naphthaleneacetate	Tetramethyl ammonium β-indoleacetate
	Tetramethyl ammonium γ-indolebutyrate

When tested for physiological effectiveness the salts were found to compare favorably with their corresponding acids and esters. The ammonium salts, however, were slightly less effective than the acids.

METHODS AND MATERIALS

Lanolin preparations containing known concentrations of the substances were used on aerial parts of the intact plants to study local acceleration or retardation of growth which causes bending and curling of stems and leaves. Water solutions were used to induce roots on cuttings, for injecting, and for soil treatments.

The following species of plants were used in the experiments: tomato (*Lycopersicon esculentum* Mill.), tropical grape (*Cissus sicyoides* L. var. *Jacquini* Planchon), *Kalanchoe daigremontiana*, privet (*Ligustrum ovalifolium* Hassk.), Jerusalem artichoke (*Helianthus tuberosus* L.), *Dahlia variabilis* Desf., Klondike cosmos (*Cosmos sulfureus* Cav.), and tobacco (*Nicotiana tabacum* L. var. Turkish).

The salts used in the experiments were prepared in this laboratory from acid growth substances or supplied by Merck and Co., Inc., Rahway, New Jersey.

The α-napthaleneacetic acid used for tests reported in earlier publications was contaminated with the beta isomer and sometimes with napthoic acid. The α-napthalene compounds used in the present tests

[1] This article was preprinted April 27, 1937.

were entirely free of the beta isomer. The use of these compounds for the present tests accounts in part for the lower minimum effective concentrations that induce bending of tomato stems and leaves, as compared with the higher minimum value previously reported (6).

RESULTS

Characteristic responses of plants to hormone-like substances have been described in previous publications (2, 6) and the details need not be

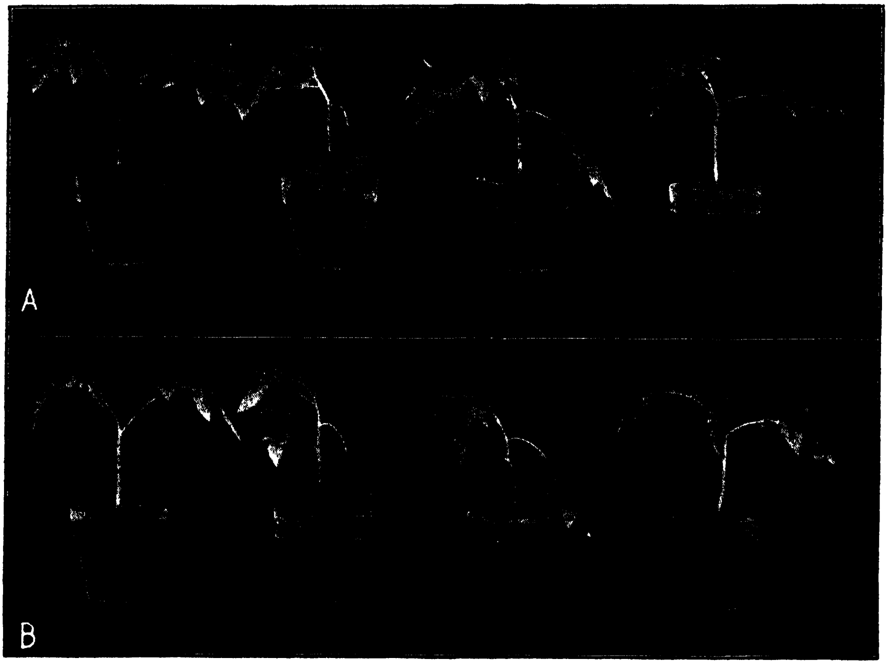


FIGURE 1. Tomato plants showing the comparative effectiveness of α-naphthaleneacetic acid (A) and potassium α-naphthaleneacetate (B) for inducing bending response. Left to right, the percentage concentrations in lanolin are as follows for both A and B: (1) Control, (2) 0.01, (3) 0.005, (4) 0.001. The preparation was applied with a glass rod on one side of the stem and on the upper side of the adjacent petiole.

repeated here. To present the results clearly, however, it will be necessary to refer to general responses.

The following responses can be induced in common by all the growth substances: local acceleration of growth causing bending of organs; swelling of treated tissues; induction of roots; systemic response (shown by epinasty of leaves) induced when substances are absorbed and distributed through the plant; retardation of growth following acceleration; and retardation of root elongation followed by swelling or increase in diameter.

To compare the physiological effectiveness of salts and acids, special attention was given to their capacity to accelerate growth locally (causing bending) and to induce roots on intact plants and cuttings.

Bending responses. Equal concentrations of the salts and their corresponding acids were dissolved in lanolin and applied in each case with a glass rod to one side of a stem and the upper side of an adjacent petiole of young tomato plants. The highest concentrations were usually 5 mg. of the substance to 1000 mg. of lanolin. Various dilutions were made until the concentration was so low that no bending of the treated parts occurred. Figure 1 shows a set of plants treated with a series of concentrations of α-naphthaleneacetic acid and the salt, potassium α-napththaleneacetate. As can be seen in the picture the degree of responses induced by the two compounds was in each case approximately equal.

Similar results were obtained with the potassium salts of indoleacetic acid, indolebutyric acid, indolepropionic acid, and phenylacetic acid.

Sodium salts prepared from naphthaleneacetic acid, indoleacetic acid, and indolebutyric acid had the same effect as the potassium salts. Salts prepared from weaker bases were also as effective as the acids. The following substitutions were made for hydrogen in the acid radicle of naphthaleneacetic acid: K, Na, Ca, Ba, Sr, NH_4, $(CH_3)_4N$, $(CH_3)_3NH$. Also ammonium, tetramethyl ammonium, and trimethyl ammonium indoleacetate and butyrate were compared with their corresponding acids. The metallic salts were approximately equal to the acids but the ammonium salts were slightly less effective.

The lower limits were determined with a series of dilutions in lanolin from 0.5 per cent down to the point where the substance was no longer effective. The following figures show the approximate lower limits:

Indoleacetic acid and the metallic salts	0.0001
Indolebutyric acid and the metallic salts	0.01
Indolepropionic acid and the metallic salts	0.01
Napthaleneacetic acid and the metallic salts	0.0001
Phenylacetic acid and the metallic salts	0.05
Ammonium salts of indoleacetic acid	0.0005
Ammonium salts of indolebutyric acid	0.05
Ammonium salts of naphthaleneacetic acid	0.0005

Potassium salts of the indole, naphthalene, and phenyl compounds induced systemic responses when water solutions of the substances were added to the soil of potted tomato, tobacco, and cosmos plants as previously reported for the acids and esters (2, 6). The response of the plants to soil treatment was evident within an hour or less as shown by bending of the stem and epinasty of leaves. Within 48 hours the stems appeared swollen and then within 6 to 10 days many roots emerged through the

bark for a considerable distance up the stem, often approaching the tip. For inducing bending responses 5 to 15 mg. of the substance in 50 cc. of water were sufficient. To induce roots along the stem a range of 10 to 40 mg. was required, depending upon the effectiveness of particular substances.

Initiation of roots. Stems and leaves of the following intact plants formed adventitious roots where treated with lanolin preparations of potassium salts listed above: dahlia, tomato, tobacco, and Jerusalem artichoke. Potassium naphthaleneacetate was the most effective root-inducing salt. This compound was fully as effective as and slightly less toxic than the corresponding acid in high concentrations. Figure 2 A illustrates adventitious roots induced on the stem of kalanchoe with a lanolin preparation containing 0.5 per cent of the potassium naphthaleneacetate. Tomato plants formed roots on stems and leaves where treated with 0.0005 per cent of potassium naphthaleneacetate or the corresponding acid. These are by far the most effective root-inducing compounds known when applied in lanolin to the aerial parts of plants. Used in this way the naphthalene compounds are approximately 100 times as effective as equal concentrations of the best indole compounds. The phenyl salt was the least effective, requiring 1.0 per cent or more.

Aerial roots of *Cissus* vines clinging to the greenhouse roof grow many feet in length without forming branch roots (5). If, however, these roots are treated with lanolin preparations of the growth substances anywhere over the region of elongation, adventitious roots are formed in a very short time. Under the most favorable conditions the new roots actually emerge within 72 hours after treatment. The response varies somewhat according to the place where the preparation is applied. Figure 2 B shows several roots treated in different ways. Considerable swelling and distortion results from treating at or close to the tip. As shown in the illustration, several roots initiated close to one another may become fasciated, growing out as a kind of wing. These monstrosities often have the appearance of a wide, flat root. These are seldom induced with low concentrations of the substances but are common when the dosage is near the upper limit for any particular substance. Not only aerial roots produced these monstrosities, but also cuttings treated with the upper limits of concentrations showed swelling and then rows of roots grown together made their appearance.

The effectiveness of the different growth substances applied to aerial roots varied in about the same order as shown for induction of adventitious roots on stems. The salts were less toxic in high concentrations than the same concentrations of the acids. The upper limits which could be used in lanolin without killing the root tissue were as follows: 1.0 per cent for potassium indoleacetate, or butyrate; 0.5 per cent for potassium naph-

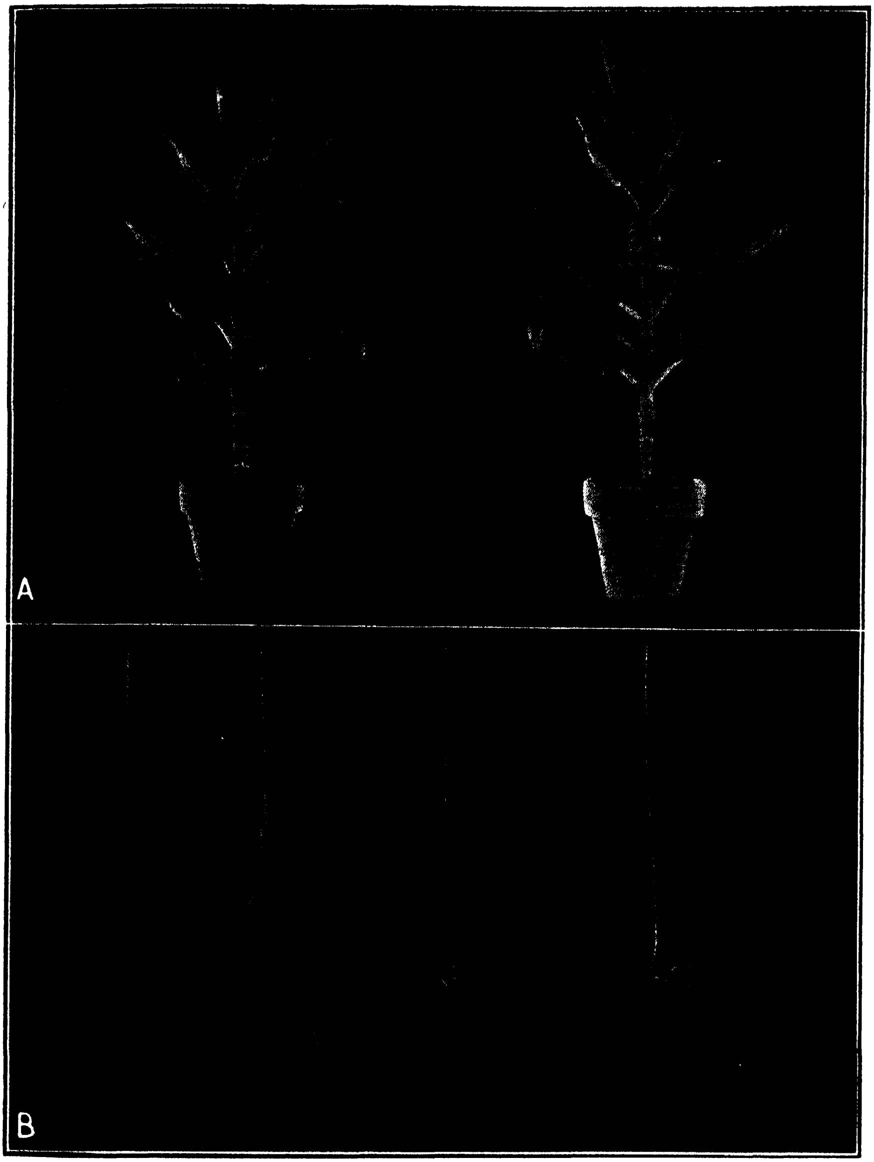


FIGURE 2. (A) Kalanchoe plants to show the root-inducing power of potassium α-naphthaleneacetate. Left, control. Right, treated around the upper part of the stem with a lanolin preparation containing 0.5 per cent of the substance. (B) Aerial roots of *Cissus* to show adventitious roots induced with lanolin preparations containing 0.5 per cent of potassium α-naphthaleneacetate. Left to right: (1) Control. (2) Treated four inches back of tip and then five days later treated at the tip to prevent inhibition of branch roots by the growing point. (3 and 4) Treated at the tip, then retreated at the tip after growth had been renewed.

thaleneacetate. The susceptibility to injury varied over the region of elongation, being greatest at the tip. The lowest concentrations which induced roots were 0.1 per cent for the indole salts and 0.01 per cent for the naphthalene salt.

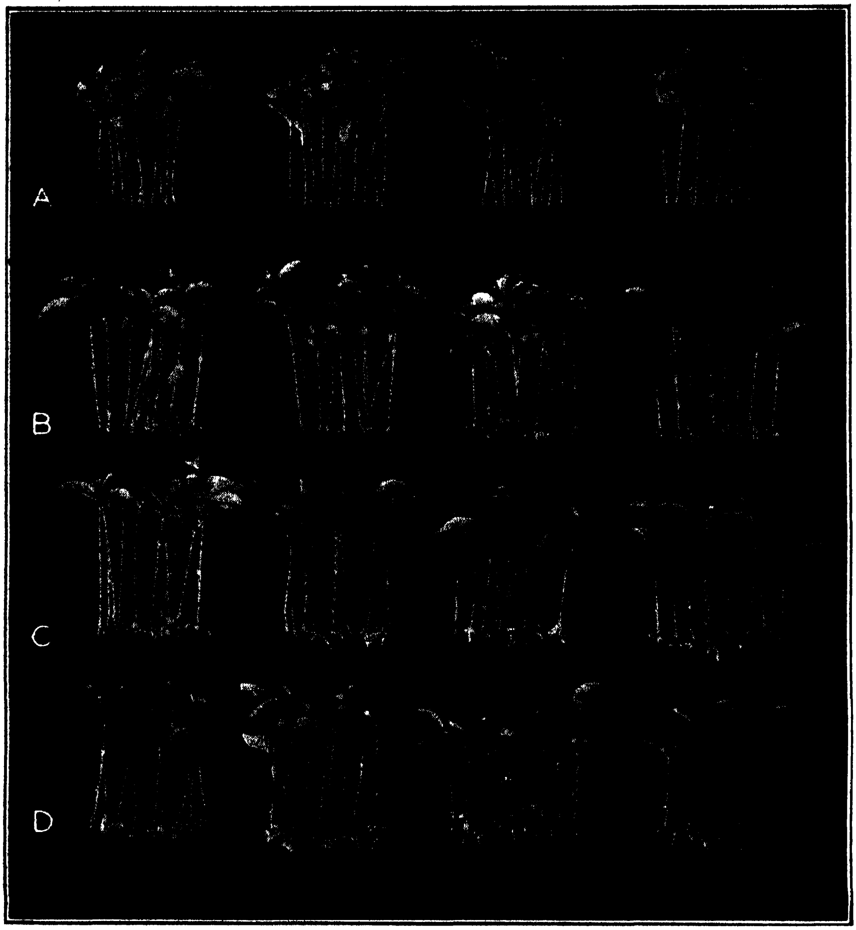


FIGURE 3. *Ligustrum* cutting showing roots induced in 14 days with water solutions of four different substances as follows: (left to right) Indolebutyric acid, potassium indolebutyrate, naphthaleneacetic acid, potassium naphthaleneacetate. The percentage concentrations are as follows: (A) Control; (B) 0.002, (C) 0.004; (D) 0.006. The basal ends of the cuttings were placed in the solutions for 24 hours, then planted in the rooting medium (peat moss and sand). The photograph was taken 15 days after treatment.

Only one treatment was necessary to initiate the roots. However, not all of the new roots continued growth. When the retarding effect was lost the main root renewed its growth and retarded further growth of short

branch roots. This appears to be a good illustration of apical dominance of a growing tip over branch roots.

Stem cuttings of woody plants were induced to root similarly by the salts and their corresponding acids when equal concentrations were used. The method consisted of standing the basal end of the cuttings in a water solution of the substance for the required time periods (16 to 24 hrs.) and then planting in the rooting medium (3). Figure 3 shows the results obtained with various concentrations of four substances. In this experiment the naphthalene compounds were more effective than the indoles. When the specimens shown in Figure 3 were carefully examined, it appeared that the salts were less toxic than the acids when the upper limits of concentration were used. If this holds in general, the salts may prove to be of considerable importance in connection with propagation of plants from cuttings.

Retardation and swelling of treated tissue. Induced bending of stems and leaves treated unilaterally with a growth substance is due to local acceleration of growth. This type of growth response is due to cell enlargement. After the first 10 to 24 hours no further bending occurs. When high dilutions of the substance are used the responding organs return toward their original position within 24 to 48 hours. However, when high concentrations are used little to no recovery may occur. In this case acceleration is followed by retardation of elongating parts and accompanied by much swelling due to both cell division and enlargement. When the material is applied near the growing tip, elongation is stopped for one to many days depending upon the concentration of the substance. Considerable retardation and swelling are usually associated with induction of adventitious roots if the substance is applied near the region of elongation of the growing organ. The acids, esters, and salts caused similar responses and in each case the effectiveness of the esters and salts approached that of the corresponding acids. The groups of substances, however, varied in effectiveness, the naphthalene compounds being by far the most active retarding substances as well as the most effective root-inducing materials.

Table I shows the comparative effectiveness of several substances in retarding elongation of aerial roots. The data show the salts less retarding than their corresponding acids.

Swelling and an increase in diameter of the root was associated with retardation of elongation (Fig. 2 B). The amount of swelling or increase in diameter varied with the region of the root treated, the substances, and the concentrations. Retardation in elongation and swelling were most pronounced when the tip of the root was treated.

As previously stated the substances accelerate growth of shoot tissue, inducing negative (away from the treated side) bending. In contrast with

TABLE I

RETARDING EFFECT OF VARIOUS SUBSTANCES APPLIED TO THE GROWING REGION OF AERIAL ROOTS OF CISSUS

Substance, % in lanolin	Number roots treated	Region treated with reference to tip	Elongation in 48 hours in inches
Control	8	—	8.25
Naphthaleneacetic acid 0.5	16	Tip	0.75
Potassium naphthaleneacetate 0.5	10	Tip	1.5
Naphthaleneacetic acid 0.5	4	3 inches back	0.75
Potassium naphthaleneacetate 0.5	5	3 inches back	1.0
Naphthaleneacetic acid 0.01	5	Tip	1.5
Potassium naphthaleneacetate 0.01	6	Tip	2.0
Indoleacetic acid 0.5	4	Tip	2.0
Potassium indoleacetate 0.5	10	Tip	2.5
Indolebutyric acid 0.5	5	Tip	3.0
Potassium indoleacetate 0.5	6	Tip	4.0
Indolebutyric acid 0.5	4	5 inches back	3.5
Potassium indolebutyrate 0.5	6	5 inches back	4.25

this type of response growth substances cause positive (toward the treated side) bending of roots. This is understood to indicate retardation of growth for roots in contrast with acceleration for shoots.

DISCUSSION

The data presented in this paper show that salts of known growth substances (acids) are physiologically active. Eight salts of practically equal effectiveness (and equal to the acid) have been prepared from α-naphthaleneacetic acid. Potassium, sodium, and ammonium salts have been prepared from three indole acids and found to be highly effective. Since there are at least 10 active acids it is reasonable to estimate that at least 80 effective salts could be prepared. Twenty-one are reported in the present paper.

The possible advantages in using salts in the applications of growth substances lie in the facts that they are more soluble in water and less toxic than the acids. Many of the effective esters are nearly insoluble and some of the acids are only slightly soluble in water. They are effective, however, in very high dilutions and soluble enough so the toxic limits can easily be reached. Studies should be made to determine the comparative ease of penetration and capacity of the various substances to spread across tissues in contrast with a tendency to follow vascular strands. Since the salts are highly ionized they may be desirable for such studies. Many qualitative differences have been noted when the same concentration of various substances was applied to comparable plants. The naphthalene compounds spread readily, causing systemic responses whereas lanolin preparations of indolebutyric compounds tend to induce a local response. Similarly if the terminal portion of a leaf of an intact

plant is immersed in a water solution containing 0.002 per cent of indolebutyric compounds the response tends to be localized at the base of the petiole. In contrast, naphthaleneacetic and indoleacetic compounds used in the same way induce a pronounced systemic effect. Indolebutyric acid or salt added to the soil of a potted plant or admitted at a cut surface induces a pronounced systemic effect.

In the search for natural hormones and synthetic compounds which induce hormone-like effects on plants, several different methods have been developed. The *Avena* method which employs the oat coleoptile as the test object is well known. This has been considered by some investigators as the standard method. To obtain reliable results with this procedure, it is necessary to use a standardized strain of oats, to maintain a constant temperature in a dark room with high humidity, and to become highly skilled in handling the equipment, conducting the experiments, and collecting the data. In fact the difficulties involved discourage all but the most persistent investigators. Another objection to the *Avena* test is that it measures only one of several growth responses induced by hormone-like substances—namely, the capacity of a substance to cause cell elongation. To satisfy all the requirements, a method should be capable of measuring cell enlargement, cell division, penetration, and the induction of new organs. At the present time no one method has been found that can fulfill all of these requirements.

The technique developed in this laboratory makes use of the tomato plant though many other species can be satisfactorily used. No special skill is required. The chemical being investigated is mixed with lanolin or olive oil and applied to one side of the stem at the region of elongation and on the upper side of the adjacent petiole. If the substance is active the leaf bends downward and the stem bends away from the leaf thus increasing the angle between the treated parts. This response occurs within one to six hours. After a compound is found effective, dilutions are made and compared with a known standard which is usually α-naphthaleneacetic acid. A concentration of one part of this substance to 1,000,000 parts of lanolin will induce epinasty of tomato leaves.

The same technique serves also to test the root-inducing capacity of a substance for intact plants but higher concentrations are required. The highly active substances induce roots over a range of concentrations beginning with 0.001 per cent and going up to the toxic limit which is usually around 1.0 per cent.

The capacity of a substance to spread throughout the plant and thus induce systemic responses is determined with the use of lanolin preparations and water solutions. The first application of lanolin preparations serves for studying induced acceleration of growth causing bending from cell enlargement, cell division, penetration, systemic response, and pro-

duction of adventitious roots. Responses involving retardation and various types of proliferation can also be determined. The water solutions are added to the soil of potted plants, injected into stems and leaves, and used for tests involving immersion of leaves. Also leafy cuttings of tomato are convenient test objects for quick response. If the basal end of a six-inch cutting is placed in a solution of an effective substance, induced epinasty of leaves becomes evident within an hour or two. To test the root-inducing capacity of substances to be used in connection with propagation of plants from cuttings, *Ligustrum* and *Euonymus* are generally used. They are usually available and both hardwood and summer cuttings respond readily. The basal ends of the cuttings are placed in water solutions of the substances for 24 hours and then planted in a rooting medium. The controls under this condition start rooting in 20 days. If the substances are effective the treated cuttings will have developed roots in 14 days (Fig. 3).

During the years of 1935 and 1936 a number of synthetic compounds which had been found by our technique to be highly effective growth substances, were given to Drs. G. S. Avery, P. R. Burkholder, and Harriet B. Creighton for their work, using the *Avena* coleoptile as a test object. The results of their work have been awaited with considerable interest since there has been some difference of opinion among scientists as to what constitutes a standard technique for determining which synthetic compounds should be recognized as growth substances. With permission of these authors some of their results (1) are included herewith so that the various methods of study can be considered together. Figure 4 presents in graphic form the results obtained by Avery, Burkholder, and Creighton with nine synthetic compounds (more are reported in their publication) applied to the *Avena* coleoptile. The data include two sets of indole compounds showing the acids in direct comparison with their corresponding methyl esters and salts. In each case the ester and salt are shown to be more effective than the acid. In an earlier publication (6) involving a different technique at Boyce Thompson Institute the same esters were reported to be more effective than the acids but the salts as shown in the present report are only equal to the acids. There appears to be, therefore, some difference in results obtained by different techniques used for comparing effectiveness of different compounds when considered from the standpoint of degree of induced activity. The main point of interest is, however, not so much the degree of activity, but the fact that both methods indicate high activity for the same substances.

The *Avena* method shows potassium indoleacetate to be twice as effective as indoleacetic acid. Inasmuch as auxin *a* has been reported by Kögl and Kostermans (4) only twice as active as indoleacetic acid, the salt and the auxin appear to be about equal in effectiveness. The methyl

ester was not as active as the salt, but was slightly better than the acid (Fig. 4). This result is interesting since esters are only slightly soluble and not ionized and yet more effective than the corresponding acid.

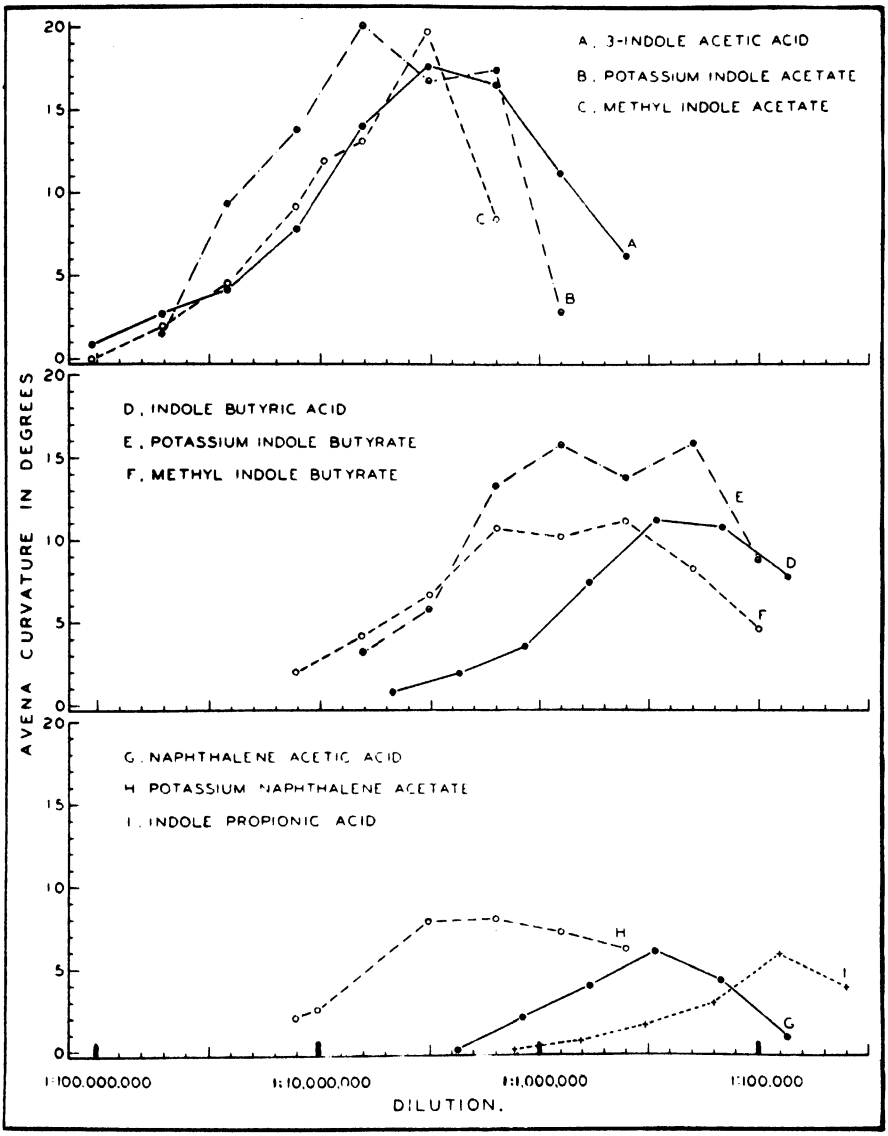


FIGURE 4. Results showing curvatures induced by nine different substances applied to the *Avena* coleoptile. These data were kindly supplied by Drs. Avery, Burkholder, and Creighton and are being published with their permission. For full details see their publication (1).

Avery, Burkholder, and Creighton (1) found also that indolebutyric acid was not as active as its salts and esters. The salt in this case is approximately three times as effective as the acid when considered from the standpoint of minimum concentrations which induce a curvature in the neighborhood of 10°. A similar order of effectiveness was found also for the naphthalene compounds, though the difference was more pronounced. Potassium naphthaleneacetate was approximately ten times as effective as naphthaleneacetic acid.

The *Avena* method consistently showed the same relative order of effectiveness for salts, esters, and their corresponding acids. Perhaps this fact is more significant than the difference in minimum concentrations required to induce a curvature of 10°. In this connection it might be well to consider the different substances from the standpoint of solubility and ionization.

Naphthaleneacetic acid (Fig. 4) as judged from the *Avena* test is comparatively ineffective. At best it induced 6° of curvature and that required a concentration of approximately 1 to 300,000. Indoleacetic acid caused a 6° curvature at a dilution of approximately 1 to 4,000,000. Thus, for inducing bending of *Avena* coleoptiles the indole acid is approximately 13 times as effective as the naphthaleneacetic acid. If, however, these two compounds are compared on the basis of their capacity to induce roots when applied to stems or leaves of intact tomato plants, α-naphthaleneacetic acid is approximately 100 times as effective as β-indoleacetic acid. For inducing bending of tomato stems and leaves, these two acids are about equal. Considered, therefore, from several angles, it seems quite clear that the *Avena* test method falls short of fulfilling our needs in determining the effectiveness of substances like naphthaleneacetic acid. The method does have, however, a distinct advantage for making a definite test with a minute amount of material. In that respect no other method approaches it. But in general no one test object can serve for testing the wide array of hormone effects. The differences in permeability of cells, capacity of substances to spread through tissue, and the power to induce anaesthesia and thus change permeability, now call for several different methods of procedure. For example, indoleacetic acid and its salts are many times as effective as indolebutyric acid in connection with bending responses but for inducing roots on cuttings the latter is the more effective. For epinastic response of tomato leaves, naphthaleneacetic acid and indoleacetic acid are about equal, but for causing curvature of coleoptiles the latter is about 13 times the more effective. However, as a root-inducing substance applied to intact plants, naphthaleneacetic acid is many times more effective than indoleacetic acid. Therefore, we need various methods and perhaps better techniques than are now known to explore the various possible effects of hormone-like substances on plants.

SUMMARY

This paper concerns a brief review of responses of plants to growth substances, a discussion of methods employed for testing purposes, and results obtained with 21 salts. The salts were prepared from the various acids previously reported as growth substances. They are as follows:

Potassium α-naphthaleneacetate
Potassium β-indoleacetate
Potassium β-indolepropionate
Potassium γ-indolebutyrate
Potassium phenylacetate
Sodium β-indoleacetate
Sodium β-indolepropionate
Sodium γ-indolebutyrate
Sodium α-naphthaleneacetate
Calcium α-naphthaleneacetate
Barium α-naphthaleneacetate
Strontium α-naphthaleneacetate
Ammonium α-naphthaleneacetate
Ammonium β-indoleacetate
Ammonium γ-indolebutyrate
Trimethyl ammonium α-naphthaleneacetate
Trimethyl ammonium β-indoleacetate
Trimethyl ammonium γ-indolebutyrate
Tetramethyl ammonium α-naphthaleneacetate
Tetramethyl ammonium β-indoleacetate
Tetramethyl ammonium γ-indolebutyrate

The salts are much more soluble than, and in general as effective as their corresponding acids.

To compare the physiological effectiveness of salts and acids, special attention was given to their capacity to accelerate growth locally (causing bending) and to induce roots on intact plants and cuttings.

An improved sample of α-naphthaleneacetic acid was found to be more effective than the acid previously reported. The new product compares favorably with indoleacetic acid for inducing bending responses and is several times as effective as the latter for inducing roots on intact plants.

Indolebutyric acid, naphthaleneacetic acid, and their salts in water solutions are the most effective root-inducing substances for cuttings.

When concentrations representing the upper limits were used, the salts were slightly less toxic than the acids as indicated by the effects on cuttings. Also the salts were slightly less retarding than the acids for growth of aerial roots of tropical grape (*Cissus*).

Aerial roots of *Cissus* which do not normally grow secondary roots were induced to form adventitious roots when treated with lanolin preparations of the various salts.

Data furnished by Avery, Burkholder, and Creighton are included, showing that several substances supplied by our laboratory were highly effective on *Avena*. They found the indole acids less effective than their salts and esters. Potassium indoleacetate was more than twice as effective as indoleacetic acid (hetero-auxin) at a dilution of 1 to 25,600,000. Naphthaleneacetic acid and indolebutyric acid, both of which are known to be powerful root-inducing substances, appeared to be only slightly effective on *Avena*. The need for several techniques and test objects is discussed.

This report shows that the acids which have been found effective as growth substances can have the hydrogen of the carboxyl group replaced by an organic or inorganic base without destroying the activity.

LITERATURE CITED

1. AVERY, G. S., JR., P. R. BURKHOLDER, and HARRIET B. CREIGHTON. Avena coleoptile curvature in relation to different concentrations of certain synthetic substances. Amer. Jour. Bot. **24**: 226–232. 1937.
2. HITCHCOCK, A. E., and P. W. ZIMMERMAN. Absorption and movement of synthetic growth substances from soil as indicated by the responses of aerial parts. Contrib. Boyce Thompson Inst. **7**: 447–476. 1935.
3. ——— Effect of growth substances on the rooting response of cuttings. Contrib Boyce Thompson Inst. **8**: 63–79. 1936.
4. KÖGL, FRITZ, und D. G. F. R. KOSTERMANS. Über die Konstitutions-Spezifität des Hetero-auxins. 16. Mitteilung über pflanzliche Wachstumsstoffe. Hoppe-Seyler's Zeitschr. Physiol. Chem. **235**: 201–216. 1935.
5. ZIMMERMAN, P. W., and A. E. HITCHCOCK. The response of roots to "root-forming" substances. Contrib. Boyce Thompson Inst. **7**: 439–445. 1935.
6. ZIMMERMAN, P. W., A. E. HITCHCOCK, and FRANK WILCOXON. Several esters as plant hormones. Contrib. Boyce Thompson Inst. **8**: 105–112. 1936.

SPRING-TREATMENT OF AUTUMN-HARVESTED GLADIOLUS CORMELS[1]

F. E. DENNY

The cormels of many varieties of gladiolus are dormant even after storage during the winter, will not germinate in the spring of the succeeding year, and need to be carried over in storage until at least the second year after harvest before good germination can be expected.

For the present experiments cormels of several of the varieties reputed to germinate with difficulty in the year succeeding harvest were obtained in order to test the effectiveness of a treatment with vapors of ethylene chlorhydrin ($CH_2Cl\ CH_2OH$), a method previously applied (1,2) to certain less dormant varieties in the period shortly after harvest.

After harvest in the autumn of 1935 the cormels were screened and those passing through a screen with square openings 0.5 inch on a side but not passing through a screen with 0.25 inch openings were used for the experiment. The cormels were divided into lots, were tied in cheesecloth bags, and were stored during the winter months under two temperature conditions: (a) at room temperature which fluctuated between about 20° and 25° C., (b) in a cold-storage room regulated closely at 10° C. The length of the storage period was from November and December 1935 to May 5, 1936, the time varying within this range with different varieties.

The cormels were treated as follows: the cheesecloth bags containing the bulblets were placed in quart-size Mason jars until the jar was nearly full; on top of the bags was placed a sheet of blotting paper to absorb any excess of liquid; for each quart jar 2 cc. of 40 per cent ethylene chlorhydrin were placed in a piece of cheesecloth 5 inches square which was then spread loosely at the top of the jar; the jar was sealed and allowed to remain at room temperature for three different periods in this experiment, i.e., 1, 2 and 4 days; the bulblets were planted in rows in the field promptly at the end of the treatment. Plantings were made in the interval from May 9 to May 14.

Germination counts were made at intervals of one to two weeks from June 22 until August 1. The crop was harvested early in October and after removal of the tops and roots the fresh weight of the corms produced from the cormels was obtained.

The results are shown in Table I. Gains in germination of cormels or yield of corms or both were obtained with all of the varieties tested. With

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 129.

Giant Nymph, Dr. F. E. Bennett, and Golden Measure (Source B) the chemical treatments gave many-fold increases after storage at either temperature; with Mrs. F. C. Peters and Minuet the treatments were successful when applied to the cormels that had been cold-stored but not when applied to those that had been stored overwinter at room temperature; with Mr. W. H. Phipps and Golden Measure (Source A) gains due to treatment were less marked when applied to cold-stored cormels since the

TABLE I

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENT OF GLADIOLUS CORMELS STORED OVERWINTER AND PLANTED IN THE SPRING

Variety	No. cormels planted in each lot	Duration of chemical treatment, days	Temperature of storage during winter			
			Room		10° C.	
			% germ. on Aug. 1	Yield of corms, g.	% germ. on Aug. 1	Yield of corms, g.
Giant Nymph	790	4	8.8	228	20.0	382
		2	9.0	120	38.0	538
		1	0.9	69	9.1	224
		Control	0.0	2	2.8	68
Mr. W. H. Phipps	730	4	34.8	446	58.5	716
		2	31.7	296	74.3	613
		1	4.9	80	21.7	352
		Control	1.8	33	15.2	300
Dr. F. E. Bennett	356	4	46.6	690	40.8	533
		2	59.1	454	75.0	478
		1	9.5	139	61.0	790
		Control	0.3	15	4.0	58
Mrs. F. C. Peters	541	4	8.8	46	23.7	149
		2	0	0	31.1	220
		1	0	0	1.1	7
		Control	0	0	0.9	8
Minuet	1210	4	0.9	16	20.0	455
		2	0.5	0	29.4	524
		1	0	0	3.1	101
		Control	0	0	7.8	62
Wilbrinck	500	4	10.6	61	7.6	31
		2	19.2	120	13.4	110
		1	2.6	11	4.6	8
		Control	1.4	4	1.8	2
Golden Measure, Source A	500	4	33.0	576	25.4	301
		2	45.0	454	37.5	528
		1	14.6	202	39.2	275
		Control	3.6	85	31.0	151
Golden Measure, Source B	407	4	43.7	222	41.3	258
		2	22.1	134	56.6	320
		1	0.7	7	12.5	20
		Control	6.6	15	11.2	25

cold treatment itself favored germination, but even under these conditions the treatment increased the yield of corms by 100 to 200 per cent; with Wilbrinck some gains due to the chemical were observed but the per cent of germination and the yield of corms were unsatisfactory, although this may have been due to low viability of the cormels rather than to a failure of the treatment. There was no doubt as to the viability of the other lots in this test, however, because in such lots as Giant Nymph, Mrs. F. C. Peters and Minuet in the room-stored series, many sprouts appeared late in September, indicating that the cormels were viable but merely too dormant to germinate in the interval from May to August.

Although storage at room temperature was unfavorable for germination as shown in columns 4 and 5 in Table I, the chemical treatment was sufficiently effective with four out of the eight lots (Dr. F. E. Bennett, Wilbrinck, and Golden Measure, Sources A and B) to overcome this condition in the cormels and to bring about a germination and yield equal to that obtained by the treatment of the less dormant cold-stored lots; with two varieties (Giant Nymph and Mr. W. H. Phipps) the results from treatments after room-temperature storage were favorable but not equal to those after cold storage; with the other varieties (Mrs. F. C. Peters and Minuet) consistent gains were obtained only by treatment following cold storage overwinter.

SUMMARY

Cormels of seven varieties of gladiolus harvested in the autumn of 1935 were stored overwinter at room temperature and at 10° C. In May 1936 the cormels were treated for 1, 2 and 4 days with vapors of ethylene chlorhydrin and planted in the field along with untreated cormels from the same stock. Germination counts made during the summer and yields of corms at harvest in the autumn of 1936 showed large gains due to the chemical treatments. Three of the varieties showed many-fold increases by treatment after storage under either temperature condition; two varieties responded to the treatment only after cold storage; and with the other lots, gains in yield due to chemical treatment following cold storage were only about 100 to 200 per cent because of the favorable effect of the cold storage condition itself on the germination of the cormels.

LITERATURE CITED

1. DENNY, F. E., and LAWRENCE P. MILLER. Hastening the germination of dormant gladiolus cormels with vapors of ethylene chlorhydrin. Contrib. Boyce Thompson Inst. **6**: 31–38. 1934.

2. ——— Storage temperatures and chemical treatments for shortening the rest period of small corms and cormels of gladiolus. Contrib. Boyce Thompson Inst. **7**: 257–265. 1935.

GERMINATION OF FRUITS OF PTELEA SPECIES

ELTORA M. SCHROEDER

INTRODUCTION

The experiments of many workers on various kinds of seeds have shown that low temperature pre-treatment overcomes dormancy in many cases. Up to the present time no extensive work has been done on seeds of *Ptelea* species. According to a progress report of the Michigan State College Experiment Station (1), seeds of *Ptelea trifoliata* planted in the greenhouse in October and December did not grow. Nursery plantings made in October gave nine per cent seedling production by June of the following year, indicating that the seeds were probably dormant. The experiments reported below were performed on *P. isophylla* Greene, *P. serrata* Small, *P. trifoliata* L., and *P. trifoliata* L. var. *mollis* T. & G., to determine the degree of dormancy in each of these species and to find a method of bringing about germination. Some workers believe that *P. isophylla* and *P. serrata* are not separate species but merely forms of *P. trifoliata*. However, the experimental results do show variation among the different species.

MATERIALS AND METHODS

The seed material used was collected at the Boyce Thompson Arboretum nursery, Yonkers, New York, on November 4, 1935, and spread to dry until November 23, 1935 when the experiment was begun. The fruit has been described as flat, dry, 2-celled, and 2-seeded, and winged all around. Cutting tests made on three lots of 100 fruits of each species showed that there were, on an average, 95.3 seeds per 100 fruits of *P. isophylla*, 102.25 seeds per 100 fruits of *P. serrata*, and 96 seeds per 100 fruits of *P. trifoliata*. Some of the fruits were empty and many were 1-seeded. Therefore, throughout the experiment 100 fruits were considered the same as 100 seeds. The wings were removed by rubbing in cheesecloth both to prevent molding and to save space.

All cultures in the ovens consisted of a mixture of moist granulated peat moss and the cleaned fruits. For germination tests, 100 fruits each of *P. isophylla* and *P. serrata* were placed in constant high temperature ovens. Others were given daily alternating temperatures so that they received 16 hours of the lower temperature and 8 hours of the higher temperature. Lots of 800 fruits of each of these two species in moist peat moss were put at 1°, 5°, and 10° C. in order to determine the effect of low temperature pre-treatment. Because of a lack of seed material, *P. trifoliata* and *P. trifoliata* var. *mollis* were given pre-treatment at 5° C. only. Sample

plantings were made monthly in a greenhouse in a mixture of equal parts of sod soil, peat, and sand. Similar lots of *P. isophylla* and *P. serrata* were put at 25° C. also, and transferred to 5° C. after one, two, three, and four months. Sample plantings from these lots were made every month after transfer to the low temperature until the seed material was used up. The greenhouse used for these sample plantings was at 21° C. during the winter and until higher outside temperatures made temperature control in the greenhouse impossible.

For outside plantings, board-covered, and mulched and board-covered cold frames with temperature ranges of 0° to 2° C. and 5° to 8° C. respectively were used. The latter frames will be referred to hereafter as the mulched frames. The 21° C. greenhouse was used as control. Duplicate lots of fruits were planted in each flat. One flat each was planted and placed directly in the mulched and board-covered frames and in the greenhouse for the duration of the experiment. Other flats, placed in the greenhouse at the same time, were transferred to the frames every month for four months, one flat going to the mulched and another to the board-covered frame. The experiment ended July 18, 1936.

RESULTS

OVEN TESTS

No Pre-treatment

Both *P. isophylla* and *P. serrata* gave very low germination percentages. *P. isophylla* had percentages ranging from 0 to 5 per cent at 15°, 20°, 25°, 30° C., and at the daily alternating temperatures of 15° to 30° and 20° to 30° C. The maximum results were 17 per cent at 10° to 20° C., and 18 per cent at 10° to 30° C. With *P. serrata*, 10° to 30° C. was best also, but with only 8 per cent. At all other temperatures the germination was less than 4 per cent. No tests were made on *P. trifoliata* and *P. trifoliata* var. *mollis* as there was not enough seed material.

Pre-treatment

Low temperature. When samples of the fruits of *P. isophylla* pre-treated at low temperature were planted in the greenhouse, those given two to four months at 1° C. and four months at 5° C. (Table I) resulted in the best stand of seedlings (86 and 81 per cent respectively), while samples from 10° C. gave only 13 per cent. Seedlings began to appear one week after planting and two weeks later the stand was complete. This was true of all species, at all temperatures, and with all treatments.

P. serrata did not show as favorable a response to low temperature treatment as *P. isophylla*. The highest seedling production (59 per cent) was obtained after five months' exposure to 1° C. (Table I). Five degrees

Centigrade was less effective (36 per cent) whereas 10° C. produced no seedlings.

P. trifoliata and *P. trifoliata* var. *mollis* were pre-treated at 5° C. only. This temperature was very effective, seedling production in the greenhouse reaching the peak after four months at this temperature (Table I).

TABLE I

EFFECT OF CONSTANT LOW TEMPERATURE AND OF DIFFERENT PERIODS AT HIGH TEMPERATURE FOLLOWED BY DIFFERENT PERIODS OF LOW TEMPERATURE ON SEEDLING PRODUCTION OF DIFFERENT PTELEA SPECIES. 100 FRUITS IN EACH SAMPLE

Species	Months at 25° C.	Low temp. ° C.	Percentage seedling production after months at low temp.						
			0	1	2	3	4	5	6
Isophylla	0	None	0						
		1		42	86	71	85	59	
		5		33	66	64	81	67	
		10		13	8	0	0	5	
	1	5		34	43	69	78		
	2	5		4	45	82			
	3	5		34	69				
	4	5		44					
Serrata	0	None	0						
		1		4	23	33	39	59	29
		5		6	10	30	36	34	24
		10		0	0	0	0	0	0
	1	5		4	16	19	48		
	2	5		9	9	28			
	3	5		1	35	12			
	4	5		22	14				
Trifoliata	0	None	6						
		5		14	80	80	102	91	
Trifoliata var. *mollis*	0	5		78	65	62	95	86	

High temperature followed by low. Samples of fruits of *P. isophylla* planted in the greenhouse after a pre-treatment period of one month at 25° C. followed by four months at 5° C., and of two months at 25° C. followed by three months at 5° C., gave about the same results (78 and 82 per cent respectively) as the constant low temperature pre-treatment (Table I). Since there is no advantage in subsequent seedling production, high temperature followed by low should not be used as the total pre-treatment period of the high plus low temperature is longer by at least one month than the constant low temperature pre-treatment.

Greenhouse sample plantings of fruits of *P. serrata* pre-treated with one month at 25° C. followed by four months at 5° C. produced the best

stand of seedlings (48 per cent). However, this was not as effective as treatment at constant 1° C. (Table I).

Since pre-treatment of fruits at constant 1° C. was slightly more effective than constant 5° C. for *P. isophylla* and definitely more effective for *P. serrata*, better results might have been obtained if the cultures at 25° C. had been transferred to 1° C. instead of to 5° C.

FLAT TESTS

Table II shows that the fruits of *P. isophylla* placed in the board-covered frame for the winter produced a better stand of seedlings (73 per cent) than those in the mulched frame (59 per cent). One or two months'

TABLE II

SEEDLING PRODUCTION OF DIFFERENT PTELEA SPECIES PLANTED IN FLATS NOVEMBER 23, 1935

Treatment in cold frames	*isophylla*	*serrata*	*trifoliata* var. *mollis*
Mulched	59	38	95
Mulched after 1 mo. in greenhouse (21° C.)	72	42	
Mulched after 2 mo. in greenhouse (21° C.)	77	47	
Mulched after 3 mo. in greenhouse (21° C.)	39	38	
Mulched after 4 mo. in greenhouse (21° C.)	25	24	
Board-covered	73	66	95
Board-covered after 1 mo. in greenhouse (21° C.)	89	64	
Board-covered after 2 mo. in greenhouse (21° C.)	82	67	
Board-covered after 3 mo. in greenhouse (21° C.)	49	27	
Board-covered after 4 mo. in greenhouse (21° C.)	15	27	
Greenhouse control (21° C.)	0	1	20

pre-treatment in the 21° C. greenhouse before transfer to the mulched frame increased the seedling production (72 and 77 per cent) until it equalled that of the board-covered frame. However, the same period in the greenhouse before transferring to the board-covered frame increased the seedling production about 5 to 15 per cent beyond that of all other treatments (82 and 89 per cent). The greenhouse control produced no seedlings.

The board-covered condition (66 per cent seedling production) was definitely more effective for *P. serrata* (Table II) than the mulched condition (38 per cent). High temperature treatment in the 21° C. greenhouse before transfer to the frames improved seedling production slightly in the mulched frame, but it had no effect on the seeds in the board-covered frame.

P. trifoliata var. *mollis* produced an excellent stand of seedlings (95 per cent) in both the mulched and the board-covered frames. At the end of the fifth month, the control flat in the 21° C. greenhouse had one per

cent seedling production. Two months later it had increased to 20 per cent.

SUMMARY

1. Oven tests of *P. isophylla* and *P. serrata* at 15°, 20°, 25°, and 30° C. and at the daily alternating temperatures of 10° to 20°, 10° to 30°, 15° to 30°, and 20° to 30° C. gave very low germination percentages.

2. *P. isophylla*, *P. serrata*, *P. trifoliata*, and *P. trifoliata* var. *mollis* are all dormant and require low temperature pre-treatment in moist granulated peat moss to break this dormancy. *P. isophylla* required two to four months at 1° C. or four months at 5° C. to break dormancy. *P. serrata* produced a good stand of seedlings in the greenhouse after five months at 1° C. *P. trifoliata* and *P. trifoliata* var. *mollis* gave excellent seedling production in the greenhouse after pre-treatment at 5° C.

3. No advantage was gained by exposure to 25° C. preceding treatment at 5° C.

4. The fruits planted in flats placed in the board-covered frame produced more seedlings than those in the mulched frame for *P. isophylla* and *P. serrata*. However, a previous period of one or two months in the 21° C. greenhouse improved seedling production in both conditions for *P. isophylla*, and in the mulched condition for *P. serrata*. *P. trifoliata* var. *mollis* produced an excellent stand of seedlings in both the mulched and the board-covered frames.

5. *Ptelea* seedlings can be produced in a practical way by planting the fruits outside in cold frames in the fall and covering the boards for the winter to prevent extreme freezing and thawing.

LITERATURE CITED

1. MICHIGAN STATE COLLEGE EXPERIMENT STATION. Progress report on study of forest tree seed, March 1926. 5 pp. 1926. (Typewritten report prepared by A. K. Chittenden and furnished through the courtesy of P. A. Herbert.)

PENICILLIUM ROT OF LILY BULBS AND ITS CONTROL BY CALCIUM HYPOCHLORITE[1]

KEITH O'LEARY AND C. E. F. GUTERMAN[2]

INTRODUCTION

At the present time the United States imports from Japan most of the lilies which are grown under glass and out-of-doors. With some of the species imported, a *Penicillium* rot of the bulbs has, for many years, been responsible for serious losses to the industry. The same rot is found on lilies shipped to this country from Europe as well as on many of our home-grown stocks. Since our largest importations of lily bulbs are from Japan and since *Penicillium* rot is so common in certain varieties shipped from that country, the present study was restricted to methods of controlling the rot in shipments from Japan. A preliminary report on earlier phases of this work has already been published (3).

SUSCEPTIBILITY

Of the lilies imported in large quantities, *Lilium auratum* Lindl. is the most susceptible to *Penicillium* rot. *Lilium rubellum* Baker and *Lilium japonicum* Thunb. are probably more susceptible, but these are imported in smaller numbers. *Lilium speciosum* Thunb. is less susceptible than *L. auratum*. *Lilium longiflorum* Thunb. is but slightly susceptible and it is only after six to eight months of cold storage that many bulbs are found to be rotted. On the European and American lilies, the rot is not as serious as with the Asiatic sorts. It is not known whether this is due to actual differences in susceptibility or to the different methods of handling.

Bulbs shipped from Japan are packed in fairly tight wooden boxes with a subsoil for the packing medium. European and American lilies are usually packed in more open boxes and the packing medium is peat or peat and sawdust. The roots are removed from Japanese bulbs before packing and the same practice is followed by most European shippers, while the American-grown varieties, in general, are shipped and stored with roots still attached.

SYMPTOMS

With *Lilium auratum*, the rot begins as small brown spots on either the scales or basal plate of the bulb (Fig. 1 A). On the scales, the spots are

[1] This work was conducted under the terms of a fellowship established and supported jointly by the Boyce Thompson Institute for Plant Research, Inc., Cornell University, The Horticultural Society of New York, and the New York Botanical Garden.

[2] Professor of Plant Pathology, Cornell University, Ithaca, New York.

warm sepia[8] colored, enlarge slowly at low temperatures, become depressed, and the centers exhibit a white mycelial growth which assumes a light dull glaucous blue color with the development of conidia. Coremia

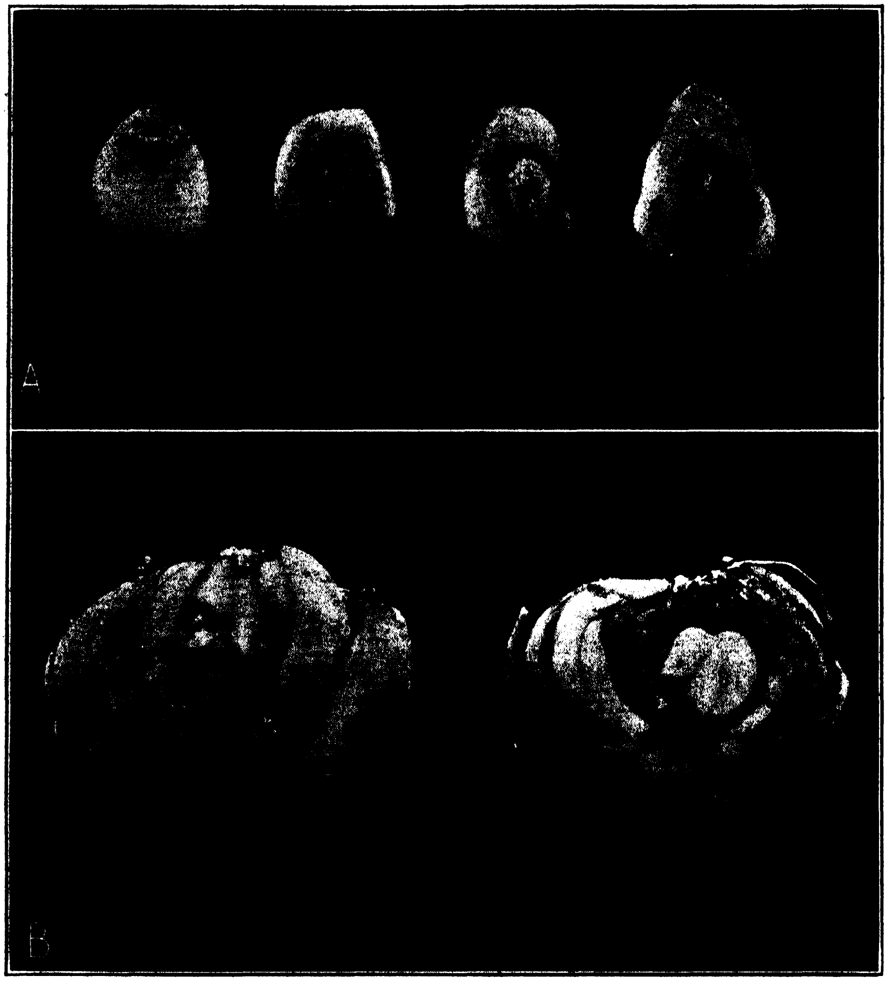


FIGURE 1. A. Scales of *Lilium auratum* showing lesions typical of *Penicillium* rot. B. *Lilium auratum* affected with *Penicillium* bulb rot. Bulb on right cut longitudinally to show internal symptoms.

may or may not be formed. The diseased area gradually enlarges to include the whole scale, and finally the basal plate or neighboring scales become infected (Fig. 1 B). The decaying areas of the scales are most frequently

[8] All color comparisons are based on Ridgway "Color Standards and Color Nomenclature."

surrounded by narrow margins which are haematite red in color. As the rot progresses, the scales, in some instances, may become chamois instead of sepia colored.

A completely rotted bulb is commonly covered with a crust of packing soil. Such bulbs, when pulled apart, disclose a layer of fruiting hyphae on the outside of each scale. Here also the color of the fruiting area is light dull glaucous blue. Coremia are often present as flattened groups giving the appearance of radiating bundles of hyphae. The tissue of the scales has now become spongy or punky and the color of the interior is pale vinaceous fawn.

The symptoms and signs on other species of lilies are practically the same except that in the cases of *L. speciosum* and *L. regale* Wilson the infected tissue remains dark even in the final stage of rot.

Bulbs which are partially rotted develop plants of varying quality depending on the location and extent of rot. If the infection is in the basal plate, the bulb usually rots after planting and fails to develop a stalk.

If the rot occurs as small areas on a few outer scales, a normal plant is produced and the bulb will be found free from rot the following autumn. *Lilium speciosum* bulbs with 3 to 5 scales rotted by *Penicillium* were compared in growth with healthy bulbs. The average height of 20 plants from healthy bulbs was 28 inches and the plants bore an average of 8.2 flowers. The average height of 20 plants from bulbs with 3 to 5 scales rotted was 30 inches and the plants bore an average of 8.5 flowers. When these bulbs were dug in the fall no *Penicillium* rot was found.

If the infection occurs in several of the inner scales near the bud, no plant is produced. Plants growing from bulbs with considerable rot are commonly chlorotic and do not attain more than one-third to one-half their normal height.

Another common rot of lily bulbs is caused by a species of *Rhizopus* (6). Bulbs rotted by this fungus are always coated with packing soil. The fungus forms sporangia on the scales and the rot is soft and wet. The rot caused by *Rhizopus* develops only at higher temperatures, while the *Penicillium* rot will develop in bulbs stored at temperatures just above freezing. Since the *Rhizopus* rot was encountered only rarely in these experiments, no data on the control of this fungus were obtained.

ETIOLOGY

Isolations have been made from various lily bulbs infected with blue mold fungi. The study of the fungi thus obtained has been confined to a series of isolates from *Lilium auratum*, *L. rubellum*, *L. japonicum*, and *L. speciosum*. The pathogenicity of the isolates was established on scales of *L. auratum* and *L. speciosum*. In these tests, scales were pulled from the bulbs and washed well in water. The scales were then soaked in a 1 to

1000 solution of bichloride of mercury for 10 minutes and rinsed in distilled water. The broken ends of the scales were sealed with paraffin and the scales then placed on dampened filter paper in moist chambers for inoculation.

Negative results were obtained when spores were placed on unwounded scales. At room temperature and when placed on wounded scales from bulbs that had been cold-stored for 9 months, the most active isolates rotted an area one inch in diameter in 10 days. On scales from freshly-harvested bulbs, the rotted area was only one-half inch in diameter in the same length of time. Isolates from *L. rubellum*, *L. japonicum*, and *L. speciosum* rotted scales of *L. auratum* at about the same rate as the isolates from *L. auratum*.

With the same isolates as sources of inoculum, the production of rot occurred much slower on scales of *L. speciosum*. It required approximately one month for these isolates to rot an area of one-quarter inch in diameter

Tests of the effect of temperature on pathogenicity showed that the fungus was able to rot bulbs at 3° C., that the optimum temperature was about 10° C., and that no rot occurred at temperatures higher than 28° C.

THE FUNGUS

Westling (9) isolated a *Penicillium* from *Lilium giganteum* Wall. which he named *P. corymbiferum*. Thom (8, p. 113) in discussing the serious losses incurred by florists who were growing bulbs of lilies, hyacinths, and related families states that "comparative study has brought recognition that the *P. cyclopium* and *P. hirsutum* series of forms are active agents in this destruction."

All but one of the isolates from the four species of lilies used in these studies are similar in morphology, cultural characters, and pathogenicity. The odd culture was isolated only once from *Lilium japonicum*. The fungus, which is usually isolated from the various species of lilies, is apparently a strain of *P. cyclopium* Westling.[4] At room temperature on Czapek's agar after five days' growth, the colonies have the following appearance: Colonies 2 to 3 cm. in diameter with a white margin of about 3 mm. The color of the center of the colony is greenish glaucous blue to Russian green. There are colorless drops of mycelial exudate on portions of the colony. The reverse color is mostly white but with pale yellow to purple tints. After several more days of growth the colony becomes zonate. The margin at this time is irregular, more or less unevenly radiating. Coremia are developed late, both at the center and margin of the colony. At 15° C. the growth is slightly slower, but more raised. With age, the

[4] The authors are indebted to Dr. Charles Thom of the United States Department of Agriculture for his very valuable help in determining the species of the fungus and for cultures of *P. cyclopium* and *P. corymbiferum* used in comparison.

cultures become deep olive gray in color. The conidiophores are rough, 3.5 to 4.2μ (measurements of 100) in diameter, vary greatly in length, and consist of a main stalk with an appressed branch. Penicillus 42 to 65μ; metulae 11.2 to 12.6μ by 3.5 to 4.2μ; sterigmata 8 to 9.8 by 2.1 to 2.5μ; conidia smooth, globose, 2.8 to 3.5μ, swelling in germination to 6 to 8μ, and usually producing one germ tube. No sclerotia or perithecia were observed.

Growth is poor on beef-peptone agar at room temperature. Plain gelatin agar is slowly liquefied. Litmus-lactose agar is colored red after five days, but with age reverses to purple. Inoculation into wounded Wealthy apples produced rot of the same type as *P. expansum* Link, but the rot was not as rapid as that of *P. expansum*.

Since Westling had originally described *P. corymbiferum* from lily, a culture of this species was secured from Dr. Charles Thom (No. 340.5035.64)[5] as well as a culture of *P. cyclopium* (No. B. 251) and these were inoculated into wounded scales of *Lilium auratum*. Both species infected the lily scales, and the rot was similar to that caused by our isolates from lilies. In rate of rotting, color of the sporulating area, and type of mycelial growth, *P. cyclopium* seemed more like the isolates in question than did *P. corymbiferum*. For a further comparison *P. cyclopium*, *P. corymbiferum*, *P. expansum*, and the *Penicillium* from lily were compared in growth on apples. *P. corymbiferum* did not attack wounded Wealthy apples, while the other three Penicillia produced rot of a similar type. The rate of rotting and appearance of spores and mycelium of *P. cyclopium* and the lily *Penicillium*, though not identical, were very similar. Thus, for the present, at least, the lily fungus is considered a strain of *P. cyclopium*. With further study of the Penicillia which attack various bulbs, some redefinitions of species will perhaps be necessary.

CONTROL[6]

In the early experiments on control, bulbs of *Lilium auratum* arriving from Japan were removed from the packing cases and those showing sign of rot were discarded. The healthy bulbs were dusted with various chemicals, after which they were repacked and held in cold storage for several months. In the first experiment, copper-lime dust (Niagara D6), sulphur dust (Kolodust), and the organic mercury dusts (Bayer 190 and Bayer P.M.A.) were used. Counts on rot after the storage period indicated that

[5] The numbers used are those which designate these cultures in the Thom collection.

[6] The authors are indebted to several commercial companies for their excellent cooperation in conducting these experiments. The experiment of 1930–31 was conducted with the cooperation of John Scheepers, Inc., of New York City; the experiment of 1931–32 with the American Bulb Company of New York City. The authors are especially grateful to the Yokohama Nursery Company of Yokohama, Japan, which has donated and treated the bulbs used in the experiments of the past four years.

organic mercuries gave some control, while copper and sulphur were ineffective. A second test with the same materials gave similar results. From these two tests, it appeared that mercuries might offer a solution to the problem. However, in the second test the mercuries caused some injury and discoloration to the bulbs.

1930–31 EXPERIMENTS

In 1930, chemicals were sent to Japan where they were applied to the bulbs before packing. This procedure assured treatment of the bulbs at or shortly after the time it was assumed that inoculation took place. The bulbs were then shipped to this country and held in cold storage for several months before inspection. In this test, the chemicals used were calomel 2 per cent, yellow oxide of mercury 2 per cent, Semesan Jr., and Dupont 693. Talc was used as the filler in preparing the calomel and yellow oxide dusts. Light applications of the fungicides were dusted on the upper and basal sides of the bulbs and any excess was shaken off.

One test of a different type was conducted during this season. The usual practice in handling most lily bulbs in Japan is to remove the roots before packing. Claims had been made by some bulb dealers that the removal of roots was one of the factors contributing to rot. To test the validity of this claim, one of two cases of untreated bulbs was packed and shipped from Japan with the roots still attached.

After cold storage for a period of four months, counts were made on the amount of rot. In all experiments reported, three classes were used in taking the data. First grade bulbs are those having no rot. Second grade bulbs are those having 1 to 5 outer scales rotted. Such bulbs will produce practically normal plants and are usually considered salable by most bulb dealers. Third grade bulbs are those with more than 5 outer scales rotted or those with rot in the basal plate or near the bud. The third grade bulbs produce either a very poor plant or no plant at all.

The results of the 1930–31 treatments for control of *Penicillium* rot are presented in Table I. Semesan Jr. and Dupont 693, organic mercury compounds, gave good control of the *Penicillium* rot. While yellow oxide of mercury was more effective than the calomel, neither of these materials gave satisfactory control. The Semesan Jr. and yellow oxide treatments caused a marked brown discoloration of the outer bulb scales. Bulbs shipped with roots attached rotted as severely as did the checks without roots. Sample lots of healthy bulbs from each of the treatments and the check were planted to determine whether or not the chemicals had produced an injurious effect on the bulbs other than discoloration. It was found that the plants from untreated bulbs were the tallest. Plants from the bulbs with roots attached were slightly shorter and all plants grown

from bulbs treated with any of the four mercury compounds were very much stunted.

TABLE I

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1930–31

Treatment	No. of 1st grade bulbs	No. of 2nd grade bulbs	No. of 3rd grade bulbs
Semesan Jr.	91	9	0
Dupont 693	91	9	0
Calomel 2%	28	63	9
Yellow oxide of mercury 2%	51	45	4
Roots on, not treated	11	80	9
Not treated	20	67	13

TABLE II

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1931–32

Control of rot Bulbs stored at 33° F. (0.5° C.) Feb. 3, 1932 Inspected Feb. 16, 1932					Growth, first grade bulbs		
Treatment	Packing case No.	No. of 1st grade bulbs	No. of 2nd grade bulbs	No. of 3rd grade bulbs	No. of bulbs planted	No. of bulbs sprouted	Average height of plants, (inches)
	1	115	4	1			
Semesan Jr.	2	116	4	0	12	9	8
	Total	231	8	1			
Yellow oxide	1	110	7	3			
of mercury	2	116	4	0	10	8	13
3%	Total	226	11	3			
	1	120	0	0			
Dupont 664	2	115	5	0	10	9	10
	Total	235	5	0			
Calomel	1	63	5	2			
3%	2	72	2	0	10	7	10
	Total	135	7	2			
	1	156	13	1			
	2	82	8	0			
Roots on, not	3	105	10	5			
treated	4	118	2	0	20	17	20
	5	59	6	5			
	Total	520	39	11			
	1	68	2	0			
	2	69	1	0			
Not treated	3	63	3	4	20	16	24
	4	58	3	9			
	Total	258	9	13			

1931–32 EXPERIMENTS

In these experiments, yellow oxide of mercury and calomel were again used, the concentration being increased to 3 per cent. Semesan Jr. was

tested once more and Dupont 664 was substituted for the Dupont 693 which had been used in the 1930–31 season. In addition, five cases of bulbs were shipped from Japan with roots still attached. The chemicals were applied in the same manner as indicated for 1930–31. Due to an unusually short period of cold storage, the amount of rot was small and hence the records give only an indication of the effectiveness of the fungicides in

TABLE III

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1932–33

Control of rot Bulbs stored at 33° F. (0.5° C.) on Feb. 6, 1933 Inspected April 28, 1933					Growth, first grade bulbs			
Treatment	Packing case No.	No. of 1st grade bulbs	No. of 2nd grade bulbs	No. of 3rd grade bulbs	No. of bulbs planted	No. of bulbs sprouted	Average height of plants, (inches)	No. of plants without buds
Semesan Bel	1 2 Total	123 141 264	177 159 336	0 0 0	20	16	6	10
Bulbs dusted with Smuttox	1 2 Total	79 21 100	206 225 431	15 54 69	20	13	9	3
360 grams Smuttox mixed with soil	1 2 Total	84 112 196	116 169 285	0 19 19	20	19	14	3
Untreated wrapper	1 2 Total	14 10 24	176 178 354	10 12 22	20	18	16	0
Copper-treated wrapper	1 2 Total	18 25 43	173 172 345	9 3 12	20	19	15	0
Mercury-treated wrapper	1 2 Total	9 21 30	179 157 336	12 22 34	20	18	6	4
Not treated	1 2 3 4 Total	91 129 57 51 328	48 57 104 126 335	51 14 39 23 127	40	36	18.1	1

controlling the fungus. The data (Table II) confirm the results of the previous experiment with bulbs shipped from Japan. Those bulbs dusted with Semesan Jr. and Dupont 664 showed slight discoloration, while the yellow-oxide-treated bulbs exhibited a more pronounced injury. Sample plantings of healthy bulbs again showed that the mercury dusts resulted in a marked stunting of the plants. This stunting was doubtless due to the

fact that root development from both the basal plate and the stem was very meagre on bulbs so treated.

The practice of leaving the roots on the bulb had no effect either in controlling rot or producing better plants.

1932–33 EXPERIMENTS

In the tests conducted during 1932–33, several new materials and methods were used. Semesan Bel, another organic mercury compound, was used as a dust on bulbs of *L. auratum* prior to packing. The same material, at a concentration of 1 pound in 7.5 gallons of water, was used as a dip for Alphie white fruit wrappers, which, after drying, were wrapped around individual bulbs at the time of packing. Other lots of bulbs were wrapped in copper-treated papers (1) which had been saturated with a solution containing 2.5 per cent copper sulphate and in untreated wrappers. Smuttox, a 4 per cent oxymethylene dust, was applied to one lot of bulbs as a dust, and in another instance, 360 grams of the material were incorporated into each bushel of the soil used as a packing medium. To avoid the loss of too much formaldehyde, the Smuttox was mixed with the soil just before the bulbs were packed.

The data (Table III) on rot were taken after 3 months' storage in this country at the usual commercial storage temperature of 33° F. (0.5° C). Semesan Bel gave the best control of all treatments used.

Sample plantings of healthy bulbs, however, showed that Semesan Bel applied either as a dust or on a treated wrapper was productive of considerable injury. Bulbs dusted with Smuttox were also injured as shown by the data on height.

1933–34 EXPERIMENTS

Using *L. auratum*, the test of copper-treated wrappers was repeated this season since the method had previously given some control without injury to the bulbs. Formaldehyde dust was also used again. In one test 360 grams of Corona oat dust (5 per cent oxymethylene) were mixed with each bushel of packing soil. In two other tests, 250 and 500 grams, respectively, of a Japanese formaldehyde dust were used with each bushel of packing soil. As in the previous season, the formaldehyde dust was mixed with the soil just before packing.

Although dips for lily bulbs had not seemed practical since their open structure would present difficulty in drying, the success of a borax dip in the control of molds of citrus fruits warranted a test on lilies. Accordingly, one lot of bulbs was dipped in 2 per cent borax solution and dried prior to packing, while another lot was handled in the same manner and, in addition, each bulb was wrapped in an untreated fruit wrapper.

The results of these tests are given in Table IV. The untreated lots showed 41 per cent third grade, 41 per cent second grade, and only 16 per cent first grade bulbs. Although the copper-treated wrapper doubled the number of first grade bulbs, the number of third grade bulbs was 19 per cent. All other treatments were inferior to the copper-treated wrappers. In subsequent growth tests, Corona oat dust, at the concentration used, resulted in visible stunting of the plants.

TABLE IV

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1933–34

Control of rot Bulbs stored at 41° F. (5° C.) Dec. 10, 1933 Inspected April 19, 1934					Growth, first grade bulbs		
Treatment	Packing case No.	No. of 1st grade bulbs	No. of 2nd grade bulbs	No. of 3rd grade bulbs	No. of bulbs planted	No. of bulbs sprouted	Average height of plants, (inches)
2% Borax dip and untreated wrapper	1 2 Total	77 33 110	75 45 120	28 47 75	40	35	19
2% Borax dip	1 2 Total	43 22 65	81 44 125	56 59 115	40	35	17
Copper-treated wrapper	1 2 Total	66 45 111	89 42 131	25 33 58	40	32	18
360 grams Corona oat dust	1 2 Total	17 34 51	106 78 184	57 14 71	40	28	4
250 grams formaldehyde dust (Japanese)	1 2 Total	26 15 41	90 53 143	64 57 121	40	33	22
500 grams formaldehyde dust (Japanese)	1 2 Total	33 19 52	90 45 135	47 56 103	40	35	28
Not treated	1 2 Total	41 8 49	82 44 126	58 68 126	40	35	19

1934–35 EXPERIMENTS

This season, 40 per cent formaldehyde solution was substituted for the formaldehyde dust. The material was mixed with the packing soil immediately before packing at the rate of 23 or 46 cc. per 50 pounds of soil. Calcium hypochlorite and naphthalene were new materials placed on test. Chlorine had been tested by Klotz (5) for the control of blue mold of citrus fruits, and while the gas was toxic to the fungus, it was also very toxic to

the fruit. Naphthalene had been tested in New Jersey (7) for the control of *Penicillium* rot of gladiolus, but without success. The calcium hypochlorite used was a Japanese product containing 24 per cent available chlorine. It was tested at three different concentrations: 20, 40, or 80 grams of calcium hypochlorite powder were mixed with each 50 pounds of packing soil immediately before packing the bulbs.

TABLE V

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1934–35

Control of rot Bulbs stored at 37° F. (2.7° C.) on Dec. 7, 1934 Inspected April 8, 1935					Growth and blooming, first grade bulbs			
Treatment	Packing case No.	No. of 1st grade bulbs	No. of 2nd grade bulbs*	No. of 3rd grade bulbs	No. of bulbs planted	No. of bulbs sprouted	Average height of plants, (inches)	Average No. of blooms per plant
23 cc. formaldehyde (40%)	1 2 Total	20 20 40	77− 77− 154	23 23 46	15	14	27.2	4.1
46 cc. formaldehyde (40%)	1 2 Total	54 45 99	56+ 66+ 122	10 9 19	15	15	32.3	4.2
20 grams calcium hypochlorite	1 2 Total	29 23 52	61 65 126	30 32 62	15	14	33	5.8
40 grams calcium hypochlorite	1 2 Total	30 34 64	54 62 116	36 24 60	15	12	25.6	4
80 grams calcium hypochlorite	1 2 Total	78 74 152	35++ 32++ 67	7 4 11	17	17	30.4	5
Naphthalene 1.25% by weight of soil	1 2 Total	46 75 121	49++ 32++ 81	5 3 8	15	14	28.5	4
Not treated	1 2 Total	17 16 33	61−− 61−− 122	42 43 85	15	14	25.5	3.8

* Signs following numbers refer to the number of rotted scales on the majority of bulbs. ++ = 1 scale rotted; + = 2 scales; no sign = 3 scales; − = 4 scales; −− = 5 scales.

After shipment from Japan and four months in cold storage, it was found (Table V) that the naphthalene and the 80 gram concentration of calcium hypochlorite had given very good control of the rot. While those bulbs treated with naphthalene were somewhat blackened by the chemical, no bulb injury occurred from the use of 80 grams of calcium hypochlorite.

The higher concentrations of formaldehyde gave some control without injury to the bulbs.

Growth tests with healthy bulbs taken from each treatment indicated that several of the treatments resulted in plants with a greater average height and an increased flower production. When the data were treated statistically by the analysis of variance method, however, these observations were not significant.

1935–36 EXPERIMENTS

In the final season's tests, the experiments were designed to obtain further information on the use of calcium hypochlorite, and more particularly to learn the effect of higher concentrations of the chemical on the bulbs and on the fungus. The concentrations used were 80, 100, and 160 grams of calcium hypochlorite powder per 50 pounds of packing soil. The bulbs were cold-stored for four and one-half months after shipment from Japan. In the untreated lots (Table VI), 24 per cent of the bulbs were first grade, 59 per cent second grade, and 15 per cent third grade. With 160 grams of calcium hypochlorite, 78 per cent of the bulbs were first grade, 20 per cent second grade, and 1 per cent third grade. Sample healthy bulbs from each treatment were grown and the data, when treated statis-

TABLE VI

TREATMENTS FOR CONTROL OF PENICILLIUM BULB ROT ON L. AURATUM; 1935–36

Control of rot Bulbs stored at 33° F. (0.5° C.) on Dec. 7, 1935 Inspected April 20, 1936					Growth and blooming, first grade bulbs			
Treatment	Packing case No.	No. of 1st grade bulbs	No. of 2nd grade bulbs*	No. of 3rd grade bulbs	No. of bulbs planted	No. of bulbs sprouted	Average height of plants, (inches)	Average No. of blooms per plant
80 grams calcium hypochlorite	1	83	35++	2				
	2	52	69++	2	20	19	27.2	5.4
	Total	135	104	4				
100 grams calcium hypochlorite	1	68	51++	1				
	2	71	44++	5	20	19	28.6	6.3
	Total	139	95	6				
160 grams calcium hypochlorite	1	95	26++	1				
	2	96	23++	1	20	19	24.6	4.8
	Total	191	49	2				
Not treated	1	16	88++	18				
	2	44	57++	19	20	18	27.1	5.3
	Total	60	145	37				

* ++ = majority of bulbs with one scale rotted.

tically, indicated no significant injury as measured by differences in height or number of blooms.

THE EFFECT OF CALCIUM HYPOCHLORITE ON BULB MITES

The bulb mite, *Rhizoglyphus echinopus* F. and R., is considered by some workers to be the cause of considerable injury to various species of lilies. Numerous measures have been suggested for the control of this pest (2, 4). In a preliminary experiment on the effect of calcium hypochlorite on bulbs of *Lilium longiflorum*, a study of the bulb mite population was made. It was found that the use of 160 grams of the chemical to 50 pounds of soil reduced the number of bulb mites from 415 per 10 bulbs in the untreated to 6 per 10 bulbs in the treated. Counts of mite population were made on ten sample bulbs of each of the treatments in the 1935–36 experiments on *L. auratum*. In making these counts, the bulbs were placed in moist chambers at room temperature for 10 days to aid in activating the mites and to promote hatching of any eggs which may have been present. No mites were found on the 10 bulbs from either the 80 or 100 gram tests. Only 2 mites were found on the 10 bulbs of the 160 gram treatment, while 115 mites were found on the 10 check bulbs. It appears from these two limited tests, that calcium hypochlorite serves as a means of control for the bulb mite as well as the *Penicillium* rot on lilies.

SUMMARY

1. The *Penicillium* rot of lily bulbs as it occurs in storage and transit has been studied largely from the point of view of control. Some pathogenicity, cultural, and morphological studies of the fungus are reported. The fungus appears to belong to the *Penicillium cyclopium* Westling group.

2. Experiments covering a period of six years are reported on control of the rot. The experiments were conducted with bulbs of *Lilium auratum*. The bulbs were handled in the manner of present commercial practice except for the treatments applied.

3. Sulphur dust, copper-lime dust, formaldehyde in liquid and dust form, and borax used as a dip gave poor control of the rot. Plain fruit wrappers or wrappers treated with copper or mercury were ineffective. The practice of removing the roots from the bulbs before packing did not seem to influence the amount of rot.

4. Organic and inorganic mercury dusts were fairly effective in controlling the rot, but mercury compounds, in general, were found to be very toxic to lilies under the conditions of these tests. While naphthalene flakes gave good control of the rot, the treatment caused discoloration of the bulbs.

5. Calcium hypochlorite powder (20 to 27 per cent available chlorine)

mixed with the packing soil at the rate of 160 grams of powder to 50 pounds of soil gave excellent control of the rot without injury to the bulbs or the plants grown from the bulbs.

6. Calcium hypochlorite, at the same concentration, was found to control the bulb mite, *Rhizoglyphus echinopus* F. and R.

LITERATURE CITED

1. COOLEY, JACQUELIN S., and J. H. CRENSHAW. Control of *Botrytis* rot of pears with chemically treated wrappers. U. S. Dept. Agric. Circ. 177. 9 pp. 1931.
2. GARVIN, PHILIP. A study of the bulb mite. Connecticut Agric. Exp. Sta. Bull. 225: 113–132. 1921.
3. GUTERMAN, CARL E. F. Final summary of the work on diseases of lilies. Hort. Soc. New York Yearbook **1930**: 51–102. 1931. (*Also in* Boyce Thompson Inst. Prof. Pap. **1** (19): 146–197. 1930.)
4. HODSON, W. E. H. The bionomics of the bulb mite, *Rhizoglyphus echinopus*, Fumouze & Robin. Bull. Ent. Res. **19**: 187–200. 1928.
5. KLOTZ, L. J. Nitrogen trichloride and other gases as fungicides. Hilgardia **10**: 27–52. 1936.
6. MASSEE, G. A lily bulb disease (*Rhizopus necans*, Mass.). Kew Bull. Misc. Inform. **1897**: 87–90.
7. *Penicillium* rot of gladiolus. Nursery Disease Notes (New Jersey Agric. Exp. Sta.) **5** (11): 1–2. 1933.
8. THOM, CHARLES. The Penicillia. 644 pp. Williams & Wilkins Co., Baltimore. 1930.
9. WESTLING, R. Ueber die grünen Spezies der Gattung Penicillium. Arkiv Bot. **11**: 1–156. 1912.

MOVEMENT OF INTRACELLULAR BODIES ASSOCIATED WITH PEACH YELLOWS

ALBERT HARTZELL

Up to the present time no intracellular bodies have been reported from peach (*Prunus persica* [L.] Stokes) tissue affected with yellows, although such bodies have been known to be associated with certain mosaic diseases in other species of plants, where they have been termed X-bodies (6, 9, 10, 12, 13, 14). It is the purpose of this paper to describe briefly the intracellular bodies found by the writer in peach tissue from trees affected with yellows, as contrasted with apparently healthy tissue in which such bodies are absent or rare. Similar intracellular bodies have been found in the salivary glands and in the cells of the intestinal wall of infected *Macropsis trimaculata* Fitch, the leafhopper vector of peach yellows, which are described here also for the first time. Comparisons are made between the intracellular bodies in this disease and similar bodies found by the writer in aster (*Callistephus chinensis* Nees.) plants affected with aster yellows, and present also in its leafhopper vector, *Cicadula sexnotata* Fall.

OBSERVATION OF INTRACELLULAR BODIES WITH THE AID OF MOTION PICTURES

The intracellular bodies in peach tissue from yellowed trees were found to move at an average rate of 1.6μ per second (50 observations). Their speed ranged from 0.5μ per second to 4μ per second, with the mode at 1.5μ to 2μ per second. Because of this comparatively great motility, methods of recording movement by means of a series of camera lucida drawings and path tracings were found unsatisfactory. It was, therefore, decided to record their activity by means of motion picture films. These films when projected on the screen by the usual motion picture projection apparatus permitted the repeated study of a particular scene and thus one could view at leisure those activities within the cell that lend themselves to investigation by this means. The films were timed to reproduce movement in each scene at approximately the same rate of speed as was observed in the microscope.

The movement of intracellular bodies was thus recorded by means of cinephotomicrography. In all, 12 scenes were photographed (Table I) representing 400 feet of film. The first six scenes were photographed by means of a camera of French make, the last six scenes by a 16 mm. Ciné-Kodak Special. The speed was 16 frames per second, magnification on the film was $\times$275 and $\times$300 in the microscope. A No. 40 Zeiss objective, numerical aperture 0.95, K10$\times$ ocular and aplanatic condenser, numerical aper-

ture 1.4, focal length 10.5 mm. were used. The sub-stage diaphragm was stopped down to 4.5 mm., resulting in an effective numerical aperture of 0.214. For illumination a 5 amp. arc light was employed. The exposure was 1/68 sec.

TABLE I

DESCRIPTION AND SOURCE OF PLANT AND ANIMAL MATERIAL FROM WHICH MOTION PICTURE FILMS WERE PHOTOGRAPHED

Scene No.	Description	Source
	Plant tissue—Peach	
1	Root tip—Healthy	Tree forced out of dormancy in greenhouse.
2	Root tip—Diseased	Tree forced out of dormancy in greenhouse. Characteristic external symptoms of peach yellows noted 3 months later.
3	Style hair—Healthy	Experimental orchard.
4	Style hair—Diseased	Yellowed tree in experimental orchard. Blossom forced out in greenhouse.
5	Petiole—Healthy	Collected in spring from experimental orchard.
6	Petiole—Diseased	Collected in spring from experimental orchard.
7	Crushed cells of petiole—Healthy	The same source as the above. Diluted with sterile water.
8	Freed intracellular bodies—Diseased	The same source as the above. Diluted with sterile water.
	Plant tissue—Aster	
9	Midrib—Healthy	Plant grown in greenhouse.
10	Midrib—Diseased	Plant infected in experimental cage.
	Insect tissue—*Macropsis trimaculata*	
11	Intestinal wall—Normal	Adult reared for 3 weeks on healthy peach seedlings.
12	Intestinal wall—Diseased	Adult reared for 3 weeks on yellowed peach seedlings.

Hand sections were made of living root tip tissue and strips of epidermis were removed from the petiole and midrib of leaves by means of a sharp razor blade, and mounted on a microscopic slide under a cover glass in distilled water. Insect tissue was mounted in Ringer's solution. Microscopic mounts of living tissue must be prepared and photographed within about 15 minutes, otherwise plasmolysis will occur and interfere with the movement of the intercellular bodies. Owing to the diffusion of light by

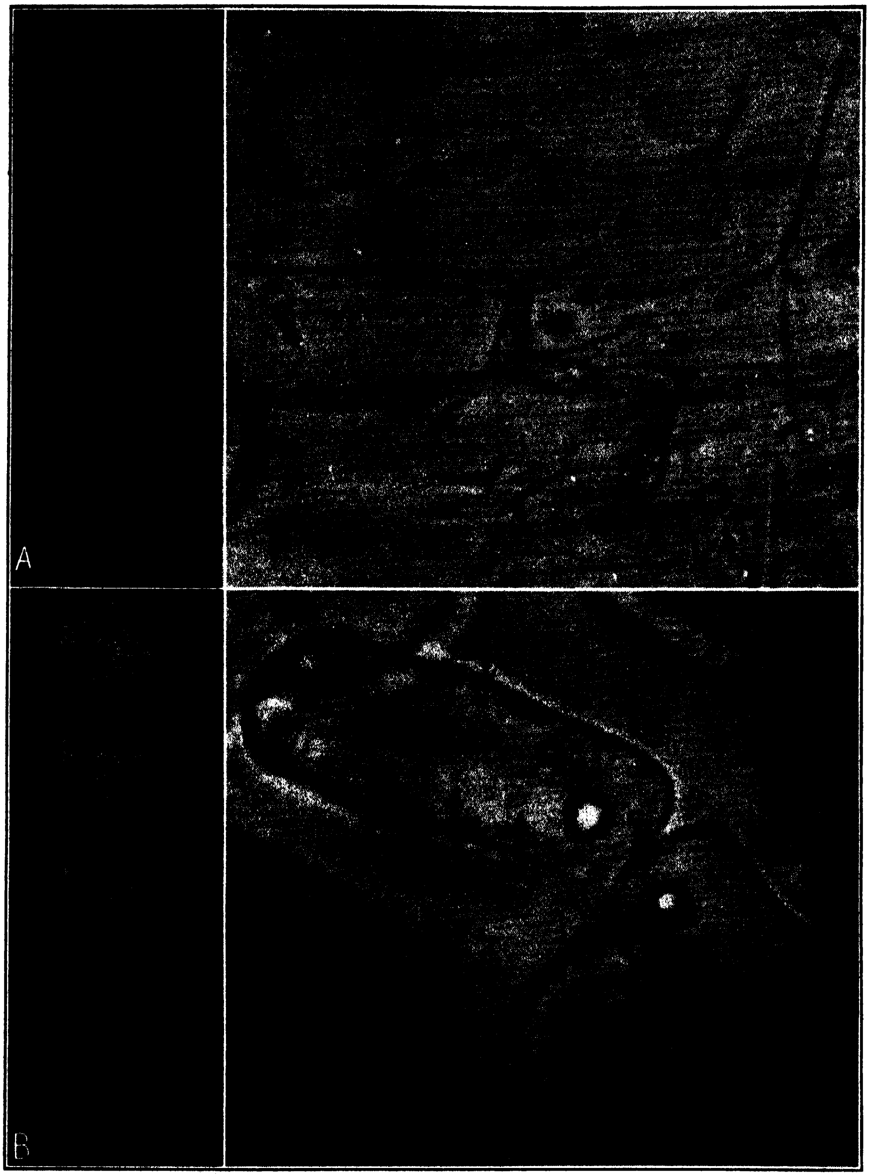


FIGURE 1. Section of a cinematographic record showing the appearance of root tip cells in peach with two corresponding enlarged frames on the right. (A) Healthy. (B) Root hair of tree infected with yellows. Note the globular intracellular bodies.

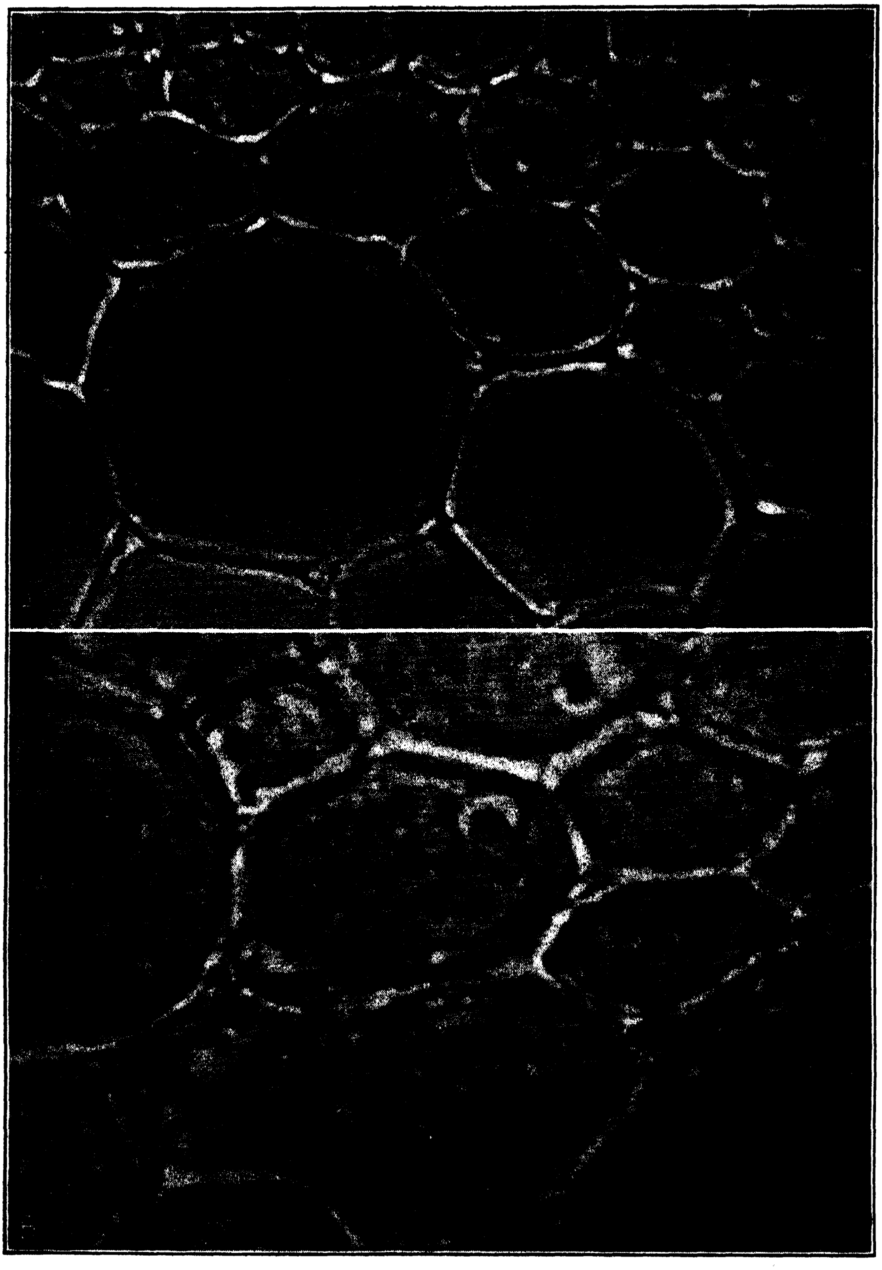


FIGURE 2. Phloem and xylem cells of healthy and diseased peach twigs. ×1660. Fixed but unstained tissue. (A) Healthy. (B) Intracellular bodies in phloem cells of peach twigs affected with peach yellows. Note the tadpole-shape.

the cytoplasm of healthy cells they were much more difficult to photograph than cells of diseased tissue. The cell juice of diseased cells appeared to be much less viscous than that in healthy cells. Blake, Cook, & Schwarze (1) have shown that juice extracted from diseased peach leaves is much less mucilaginous than juice extracted from the leaves of healthy plants.

Microscopic examination of living tissue from diseased peach trees showed rapidly moving bodies in the cells. In root tip tissue (Fig. 1) these bodies were observed at least three months before the characteristic leaf and twig symptoms of this disease were noted. Intracellular bodies were observed in style hairs of blossoms from diseased trees as soon as the flower buds opened. Style hairs are well suited for study since the cell wall is transparent permitting observation of the cellular contents without disturbing the activity within the cell.

Similar bodies were observed in the cells of the midrib of aster plants infected with aster yellows.

DESCRIPTION OF INTRACELLULAR BODIES IN THE PLANT

When the intracellular bodies are examined at a magnification of 400, they appear to be globular. That they are not perfect spheres is evident by difference in refraction as they rotate in the living cell. When examined in unstained fixed tissue at a magnification of 2300, they were found to be tadpole-shaped, the tail-like projection not being visible at low magnifications (Fig. 2). In size they range from barely visible to 3μ in length, the majority observed ranging from 1 to 3μ. They resemble rounded bits of cytoplasm. They may occur singly in a cell or, in advanced stages of the disease, 50 or more bodies may be observed in a single cell. The intracellular bodies do not seem to maintain any definite spacial relationship with the cell nucleus. They appear to be moving for the most part in the cell sap of the vacuole. The movement does not seem to be due entirely to the streaming of the protoplasm within the cell, as in a number of cases the bodies were observed moving in the direction opposite to the protoplasmic stream.

In order to study more carefully the movement of the intracellular bodies, motion picture scenes were projected on cross section paper. By this means attention could be centered on a particular body, its direction of movement indicated by means of an arrow and pencil marks made at the points where the body changed direction (Fig. 3 A and B).

When intracellular bodies were released from the cells by crushing cells of petiole tissue from peach leaves of yellowed trees on microscopic slides in sterile water, they still retained their power of movement. Crushed cellular contents from comparable healthy tissue did not show the same freedom of movement as did the intracellular bodies of diseased tissue

Similar diagrams were made of the movement of cellular contents from crushed diseased and healthy cells (Fig. 3 C and D).

The diagrams described below were compared with similar diagrams made in the study of Brownian movement. The zig-zag course followed by the intracellular bodies is suggestive of Brownian movement. But that the movement of the bodies is not a Brownian movement is shown by the following experiment. Style hairs of blossoms from yellowed peach trees were suspended in a hanging drop culture in a modified Van Tieghem cell and a current of gas consisting of 60 per cent carbon dioxide and 20 per cent oxygen was passed through the cell. In a few minutes both the movement of the intracellular bodies and streaming of the cytoplasm ceased. The motility of the bodies and the streaming of the cytoplasm could be restored by drawing a current of air through the Van Tieghem cell. Soil solutions containing particles in active Brownian movement when exposed in a Van Tieghem cell to similar concentrations of CO_2 and O_2 were not affected, the characteristic movement of the particles continuing. This is evidence against the view that the motion of the intracellular bodies in the living cell is merely a matter of Brownian movement.

Tests made with vital stains indicate that the intracellular bodies are made up of living cytoplasm containing protein. Failure of brilliant cresyl blue, eosine, iodine, and methylene blue to stain deeply suggests that the bodies possess a living membrane. Flagellate stains such as Giemsa, azure eosine, brilliant cresyl blue, and dilute India ink (2) indicate the presence of a membrane that undulates and of a whip-like projection at one end of the body. These are not visible except at the highest magnifications. The intracellular bodies did not stain with iodine nor with Sudan III, indicating that they are not starch grains nor fat globules.

In the preparation of materials killed and fixed in the usual botanical fixatives and stained with Flemming's triple stain the first evidence of the disease was the clouding of the epidermis (Fig. 4 A and B). Leaves were collected in 80 per cent alcohol at intervals of two weeks throughout the growing season from peach seedlings that had been budded with scions from yellowed trees. The next symptom to be observed was the appearance of vacuoles with dark stained margins. Within the vacuoles were small particles with radiating strands. Later the smaller particles coalesced to form larger particles. Plasmodia-like bodies appeared in the conductive tissues (Fig. 4 C and D). They filled a whole cell or there were several bodies in a single cell. The phloem cells were greatly enlarged. Finally, plasmodia-like bodies appeared in the palisade and parenchyma cells (Fig. 5 A and B). These cells together with their plastids were greatly reduced in size (3) with large interspaces between cells. The tissue was brittle and broke up when sectioned.

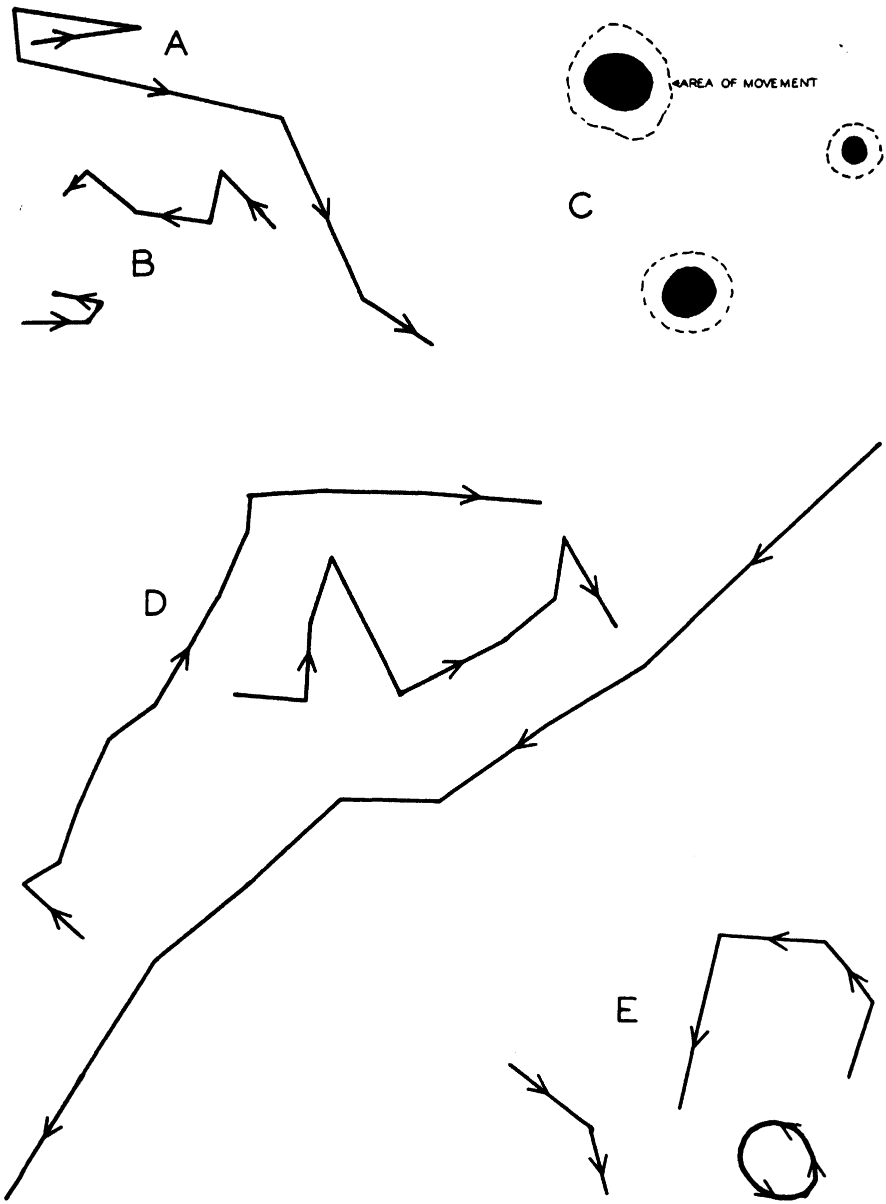


FIGURE 3. Diagrams of paths of intracellular bodies in peach tissue and infected insect (*Macropsis trimaculata*) tissue. (A) In style hair cell infected with peach yellows. (B) In petiole cell infected with peach yellows. (C) Cellular contents from crushed cells of healthy leaf petiole. (D) Freed intracellular bodies from petiole of crushed leaf from tree affected with peach yellows. (E) In cell of intestinal wall of *Macropsis trimaculata* which had fed on tree affected with peach yellows.

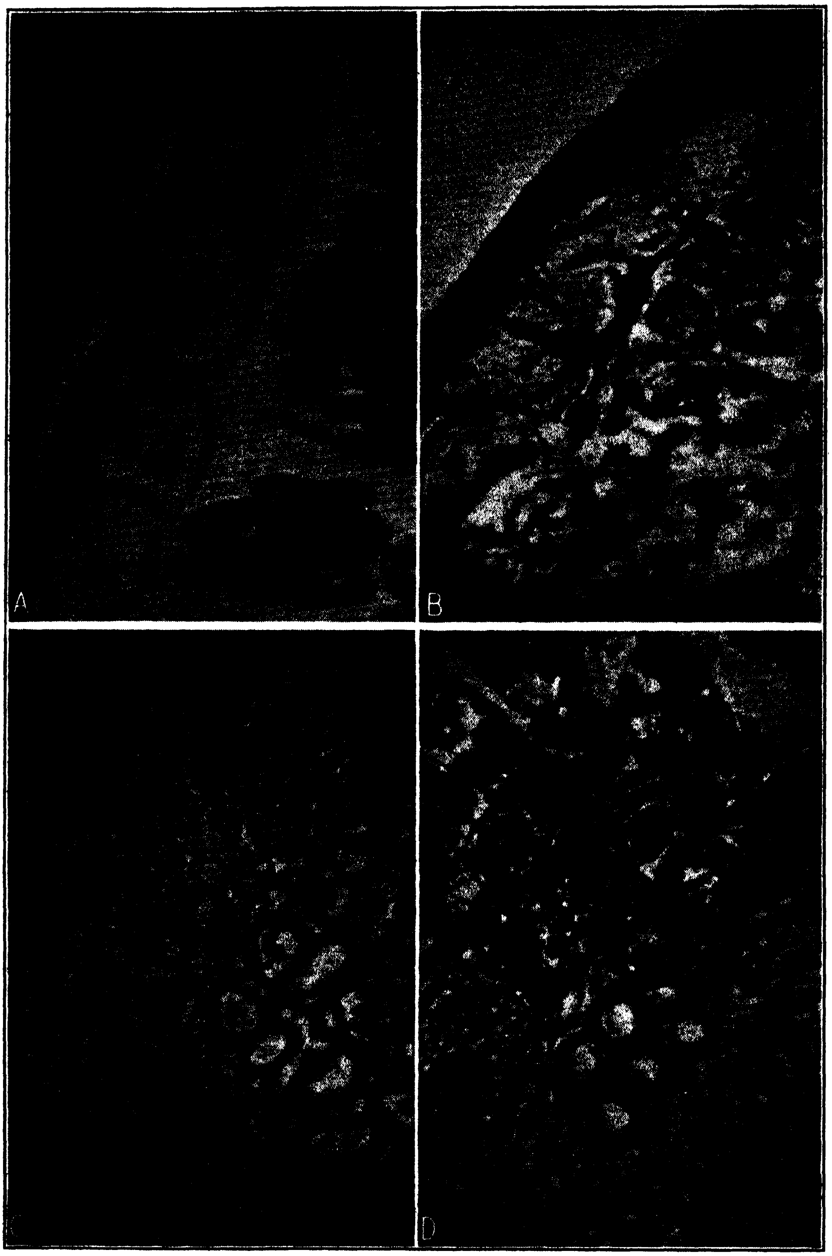


FIGURE 4. Stained and fixed sections of tissue of peach trees budded with yellowed scions, showing early stages in the development of the disease. $\times 1040$. (A) Epidermis of healthy leaf. (B) Epidermis of leaf from diseased tree. Note the clouding of the cells. (C) Conductive tissue of healthy leaf. (D) Conductive tissue of leaf from diseased tree. Note enlargement of the cells of phloem and presence of plasmodia-like bodies.

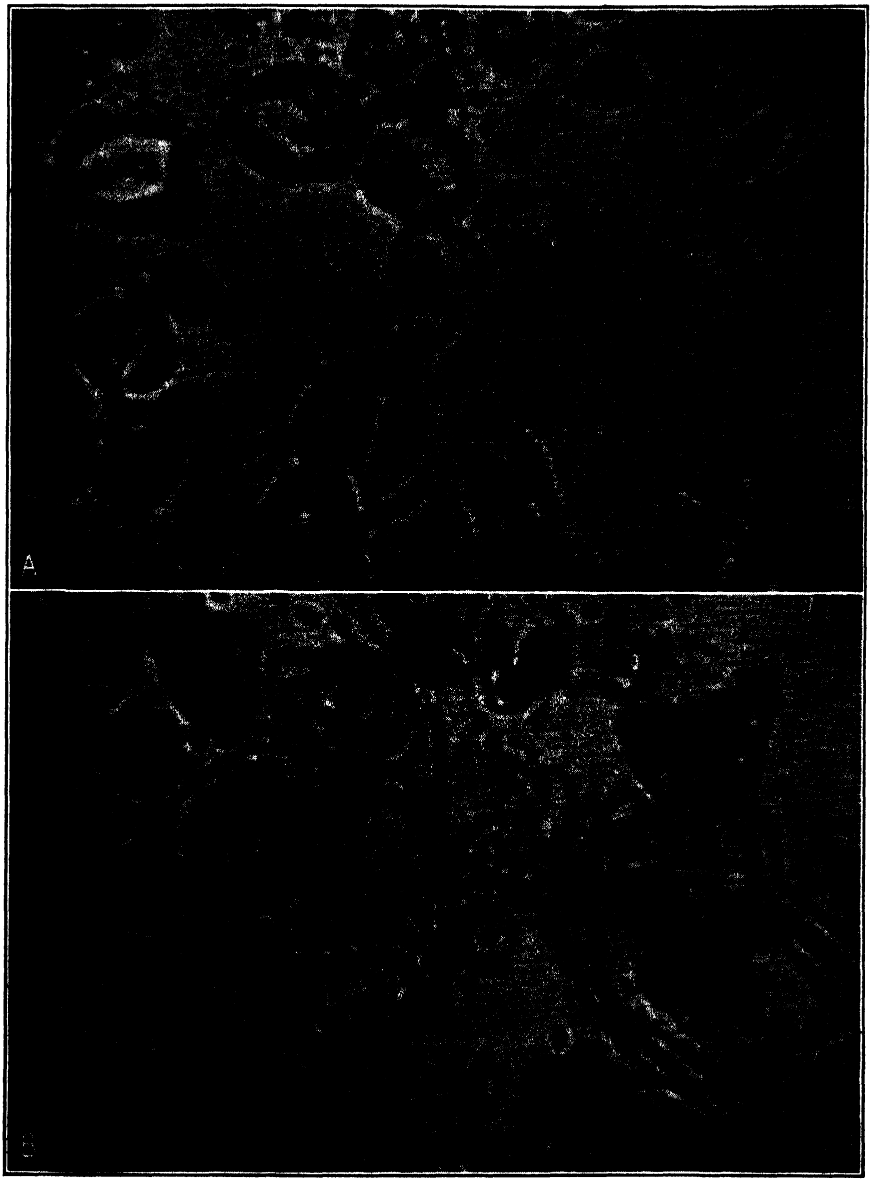


FIGURE 5. Stained and fixed sections of tissue of peach trees budded with yellowed scions, showing final stages in development of the disease. Mid-section of leaf: (A) Healthy. (B) Diseased. Note presence of vacuolated bodies in cells. ×1650.

DESCRIPTION OF INTRACELLULAR BODIES IN THE INSECT VECTOR

Motile intracellular bodies were first observed during the summer of 1936 in cells of the intestinal wall of vivisected *Macropsis trimaculata* leafhoppers that had been caged on yellowed trees for 22 days. Motion picture films of this material showed that the bodies were in such rapid agitation that they appeared to give a quivering motion to the entire tissue.

Vivisections of adult leafhoppers were made with the aid of a binocular dissecting microscope. The insect was impaled on a microscopic slide by running liquid paraffin around it and allowing the paraffin to harden. The tissue was teased apart in a drop of Ringer's solution by means of *minuten nadeln* that had been fused into glass rods to serve as handles. Bits of tissue dissected from the salivary glands and the intestinal wall were removed to microscopic slides in drops of Ringer's solution for examination under the compound microscope.

Measurements made of the intracellular bodies showed them to range in size from barely visible to 1μ in diameter. Their paths were plotted as described above for plant tissue (Fig. 3 E). Dissections made from adults caged on healthy trees for a like period showed no intracellular bodies. The tissue from the intestinal wall of normal leafhoppers was much more dense than from infected leafhoppers. Adults that had been fed on yellowed twigs for eight days showed similar motile intracellular bodies in the salivary glands. When the bodies were freed from the cells, in the cell juice diluted with Ringer's solution, they still retained their power to move. The salivary glands of leafhoppers that had been reared on healthy trees did not show evidence of intracellular bodies nor rapid moving particles. Intracellular bodies were found also in the cells of the intestinal wall of *Cicadula sexnotata* adults from colonies of leafhoppers known to transmit aster yellows. The larger bodies were about 1μ in diameter. Infected leafhoppers from such colonies apparently retained their infectivity for at least a month, as individuals caged separately on healthy aster plants transmitted aster yellows to these plants in about two weeks. Leafhoppers from non-infective colonies did not have intracellular bodies in the cells of the intestinal wall, nor did individuals from such colonies transmit aster yellows when allowed to feed on healthy plants.

Fixed insect sections were prepared from whole mounts of the leafhoppers which had been killed in 95 per cent alcohol. Incisions were made in the thorax and abdomen to permit rapid penetration of the alcohol. The tissue was dehydrated in absolute alcohol and imbedded in paraffin after running through xylol. Gradual transitions were made by using combinations of alcohol and xylol and paraffin and xylol in order to avoid unnecessary tearing of the tissue due to the fixatives. Sections were cut 5μ thick. Microscopic examination of *unstained* fixed tissue showed the presence of intracellular bodies, which were globular and surrounded by

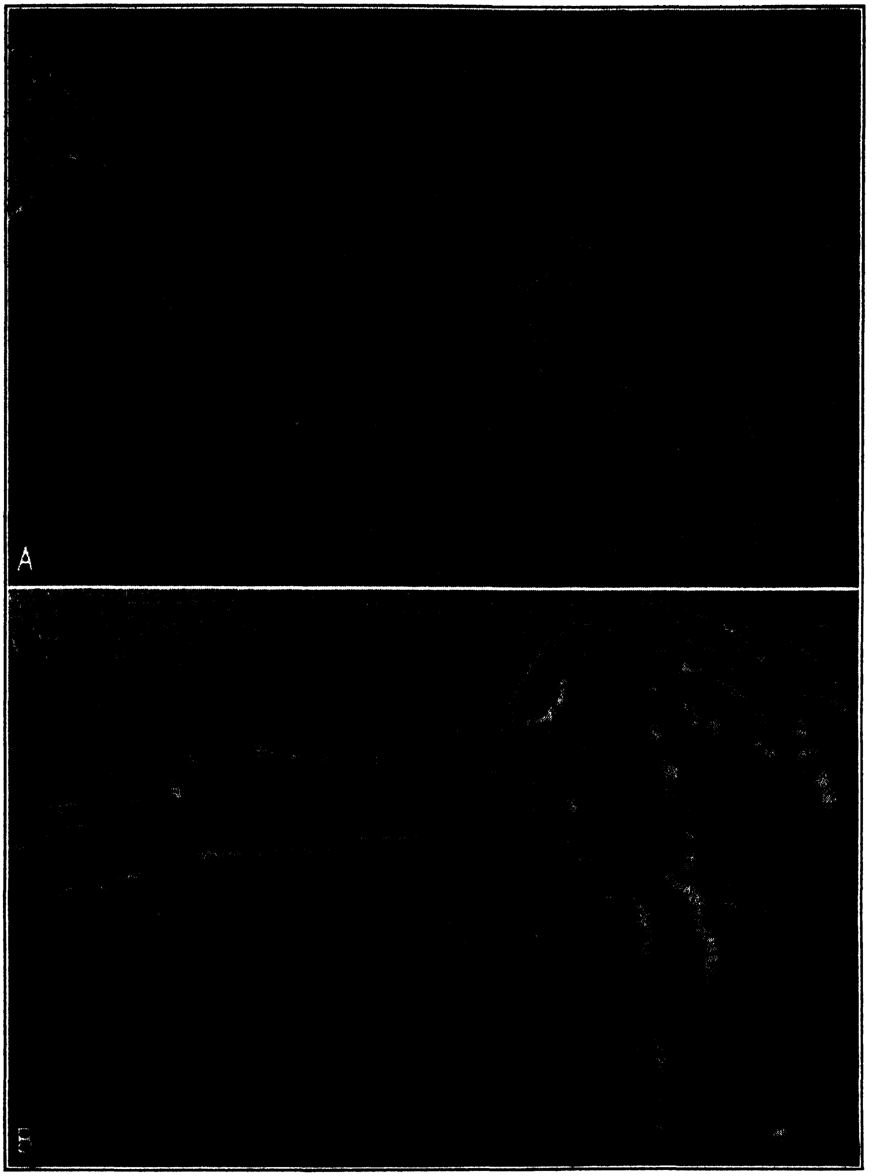


FIGURE 6. Fixed *unstained* tissue of salivary glands of *Macropsis trimaculata*. ×1690. (A) Normal tissue. (B) Intracellular bodies from adult leafhopper that had fed on peach tree infected with yellows. Note the dark globular bodies surrounded by halos.

hyaline halos (Fig. 6). When the tissue was stained with Heidenhain's iron-alum haematoxylin the outlines of the bodies were obscured. Failure of other workers to examine carefully microscopically the living tissue and unstained preparations of fixed tissue of the salivary glands and digestive tract of infective leafhoppers, is believed to be the reason why similar bodies have not been observed previously (5).

COMPARISON OF INCLUSION BODIES ASSOCIATED WITH OTHER DISEASES

With the limited information available it is not possible to give any definite interpretation as to the exact nature of the intracellular bodies observed in this investigation. The above studies show clearly that they are not artifacts. While it is possible that the bodies may be organisms it is equally plausible to believe that they are the resulting products of the reaction of the host cells to the disease. They may possibly be degeneration products of the diseased cell. No crystals were found associated with them as has been observed in tobacco mosaic. They also differ from X-bodies in that they move more rapidly and are not amoeboid. The bodies resemble in general the cellular inclusions (4, 7) of unknown nature associated with certain of the virus diseases of animals such as smallpox and rabies. They do not resemble the polyhedral bodies (8) of insects as they are not hexagonal in outline and when crushed on a slide do not fragment into angular particles as has been described for that disease. Fixed and stained leaf tissue from trees having peach yellows bears a superficial resemblance to histological preparations of fowl-pox (11).[1]

SUMMARY

The movement of intracellular bodies found by the writer associated with peach yellows was recorded by means of cinephotomicrography. Scenes have been prepared showing these bodies moving in the cells of living peach leaf petiole, style hair and root hair tissues, as contrasted with apparently healthy tissues in which such bodies are absent or rare.

Intracellular bodies similar in appearance were found in the cells of the intestinal wall and salivary glands of living *Macropsis trimaculata*, the leafhopper vector of peach yellows, which had fed from one to three weeks on yellowed trees. These were recorded also by means of motion pictures. Similar bodies were not found in corresponding tissues in leafhoppers that were reared on healthy trees. A parallel relationship seems to hold for aster yellows and its insect vector. In general there was much more cellular disturbance observed in the tissues of infected plants and insects than was

[1] The writer is indebted to Dr. A. M. Pappenheimer of the College of Physicians and Surgeons, New York, New York, for the privilege of examining histological preparations of inclusion bodies commonly associated with animal diseases.

found in corresponding normal tissues. Whether the intracellular bodies in the infected leafhoppers bear a primary relationship to those found in diseased plant tissues or are secondary in nature, was not determined.

When infected tissue was crushed on microscopic slides and the intracellular bodies were released into the cell juice, those from infected peach leaf petiole tissue and from the cells of the digestive system of infected leafhoppers showed marked motility. Whether the movement of the intracellular bodies observed is due to vital activity or to purely physical forces has not been definitely determined. Infected style hairs mounted in a hanging drop culture in a Van Tieghem cell when examined under the microscope showed motile intracellular bodies and streaming cytoplasm. When a current of gas consisting of 60 per cent carbon dioxide and 20 per cent oxygen was passed through the cell both the movement of the intracellular bodies and the streaming of the cytoplasm ceased. The motility of the bodies and the cytoplasmic stream could be restored by drawing a current of air through the cell. Solutions consisting of non-living material similarly treated showed no effect on Brownian movement. This would suggest that the motion of the intracellular bodies is not Brownian movement.

LITERATURE CITED

1. BLAKE, M. A., MEL. T. COOK, and C. A. SCHWARZE. Studies on peach yellows and little peach. Phytopath. **7**: 76–77. 1917.
2. COLES, ALFRED C. Staining and microscopical demonstration of filterable viruses. Jour. Roy. Microscop. Soc. **55**: 249–255. 1935.
3. COOK, MEL. T. Peach yellows and little peach. Bot. Gaz. **72**: 250–255. 1921.
4. COWDRY, EDMUND V. Intracellular pathology in virus diseases. *In* Rivers, Thomas M., editor. Filterable viruses. p. 113–154. Williams & Wilkins Co., Baltimore. 1928.
5. DOBROSCKY, IRENE D. Morphological and cytological studies on the salivary glands and alimentary tract of *Cicadula sexnotata* (Fallen), the carrier of aster yellows virus. Contrib. Boyce Thompson Inst. **3**: 39–58. 1931.
6. ECKERSON, SOPHIA H. An organism of tobacco mosaic. Bot. Gaz. **81**: 204–209. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 109–114. 1926.)
7. FINDLAY, G. M., and R. L. LUDFORD. The ultramicroscopic viruses: I.—Cell inclusions associated with certain ultramicroscopic diseases. A pictographic review. British Jour. Exp. Path. **7**: 223–225. 1926.
8. GLASER, RUDOLF W. Virus diseases of insects. Part II. The polyhedral diseases. *In* Rivers, Thomas M., editor. Filterable viruses. p. 301–334. Williams & Wilkins Co., Baltimore. 1928.
9. GOLDSTEIN, BESSIE. Cytological study of living cells of tobacco plants affected with mosaic disease. Bull. Torrey Bot. Club **51**: 261–274. 1924.
10. ——— A cytological study of leaves and growing points of healthy and mosaic diseased tobacco plants. Bull. Torrey Bot. Club **53**: 499–600. 1926.
11. GOODPASTURE, ERNEST W. Virus diseases of fowls as exemplified by contagious epithelioma (fowl-pox) of chickens and pigeons. *In* Rivers, Thomas M., editor. Filterable viruses. p. 235–278. Williams & Wilkins Co., Baltimore. 1928.
12. IWANOWSKI, D. Über die Mosaikkrankheit der Tabakspflanze. Zeitschr. Pflanzenkrankh. **13**: 1–41. 1903.

13. MCKINNEY, HAROLD H., SOPHIA H. ECKERSON, and ROBERT W. WEBB. The intracellular bodies associated with the rosette disease and a mosaiclike leaf mottling of wheat. Jour. Agric. Res. **26**: 605–608. 1923.
14. PALM, B. T. De Mozaiekziekte van de Tabak een Chlamydozoonose? (Voorloopige mededeeling.) [Is the mosaic disease of tobacco a chlamydozoonose? (Preliminary note.)] Bull. Deliproefstat. te Medan-Sumatra. No. 15. p. 1–16. 1922. (*Abstr. in* Rev. App. Mycol. **1**: 394–396. 1922.)

IDENTIFICATION OF CRYSTALLINE CELLULOSE IN YOUNG COTTON FIBERS BY X-RAY DIFFRACTION ANALYSIS[1]

Wayne A. Sisson[2]

The average period of cotton fiber development is approximately 50 days. One of the substances elaborated by the living protoplasm of the fiber and used for wall formation is cellulose. There is no definite agreement concerning the time of the first appearance of cellulose in the young fiber. Farr and Eckerson (6) find uniform-sized ellipsoid particles of cellulose in the living cytoplasm of the epidermal cotton cell at the time of initation of fiber growth. These cellulose particles are covered with a coat of non-cellulosic material. Earlier workers (13) find no evidence of cellulose until after the sixteenth day, when it is first deposited as a thin "network" inside the original limiting membrane. These conclusions (6, 13) are based on microscopic and microchemical data.

X-ray data published to date (1, 2, 9, 12) have shown no evidence of cellulose until after the thirtieth day. Clark, Farr, and Pickett (1, 2) examined with X-rays four untreated samples (18, 21, 35, and 50 days) and found a crystalline substance which increased in micellar size and decreased in unit cell dimensions with growth, as indicated by a change in the diffraction ring breadth and diameter. The usual cellulose pattern was obtained at 35 days. Hess, Trogus, and Wergin (9, 12) in their examination of moist untreated samples, ranging in age from 24 to 50 days, were able to distinguish two crystalline constituents: one, called the "primärsubstanz," is present during cell elongation; the other, crystalline cellulose, appears only after the completion of elongation, or when the secondary thickening of the wall begins. They showed that the change in crystalline diagram of the fiber with age is not due to a change in unit cell dimensions of cellulose, but to the presence of the non-cellulosic "primärsubstanz," the pattern of which is gradually displaced by that of cellulose as the fiber cell wall thickens. This gives an illusory change in the diameter and width of the diffraction ring. They found no X-ray evidence of crystalline cellulose below 36 days.

The X-ray investigation (1, 2) of the daily stages of cotton fiber growth was started, in cooperation with microscopic studies by Farr (4), during

[1] Presented in part before the Division of Cellulose Chemistry at the 92nd meeting of the American Chemical Society, Pittsburgh, Pennsylvania, September 7–11, 1936, under the title "The effect of certain non-cellulosic constituents on the X-ray diagram of cellulose."

[2] Cellulose Department, Chemical Foundation, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

the winter of 1929–30 at the University of Illinois.[3] More recently, in order to determine why X-ray and microscopic data (6) did not correlate, the earlier work was repeated and the investigation extended to include the treatment of young fibers with chemical reagents commonly used for the purification or preparation of standard cellulose. In the present paper it will be shown that the age at which cellulose is detected by X-ray analysis depends on the past history and pretreatment of the sample, and that under favorable experimental conditions there is good agreement between X-ray and microscopic data (6) at all periods of fiber growth.

MATERIALS AND METHODS

Both field and greenhouse grown cotton fibers were used for the present study. The field material was *Gossypium hirsutum* L. Strain 4 of a representative pure bred, American cotton described by Farr (4). The majority of experiments, however, were made on greenhouse samples of the same species grown at the Boyce Thompson Institute at various intervals during 1932–36. The conditions of growth for both field (4) and greenhouse plants (5) and the method of collecting the samples have been described. Two additional varieties, B-1 and Acala,[4] on which the earlier experiments were repeated, were grown in the greenhouse during 1936. The results reported in this paper apply to all three varieties.

The flowers were marked with dated tags at the first day of flowering, and at the end of 50 days bolls of every daily stage from flowering to maturity had been obtained. Bolls of a definite age were collected, but they were not from a selected position on the plant, or from plants of a definite age. Likewise, in preparing the samples, no special precaution was taken to select fibers from a particular position on the seed, or seeds from a definite location on the boll. The variation due to these factors, however, was decreased by the large sample, consisting of mixed fibers from five to ten different bolls, which was first collected and later divided into aliquot parts. The collected samples were prepared immediately for X-ray examination, or they were preserved until ready for use. The preserved fibers attached to the seeds were kept in a solution of 1.4 per cent formalin and 70 per cent ethyl alcohol. Microscopic examination (4) showed this method of preservation to produce no appreciable distortion or change in the fiber structure after two years.

[3] At the suggestion of Professor Clark in 1932 the X-ray work was extended to a more complete series of untreated samples, and later to fibers extracted in organic solvents. This work was carried out while the writer was a Textile Foundation Fellow at the University of Illinois, Urbana, Illinois, and later as Associate Cotton Technologist, Division of Cotton Marketing, Bureau of Agricultural Economics, U. S. Department of Agriculture, Washington, D. C.

[4] Cotton seeds kindly furnished by Prof. R. Y. Winters, North Carolina State College, Raleigh, North Carolina.

The untreated dry samples were prepared for X-ray examination from both fresh and preserved material by carefully dissecting the fibers from the seeds and placing them on a glass slide to dry at room temperature. The absence of seed coat fragments in the dissected samples was confirmed by microscopic examination . All samples for the present study consisted of fibers in random arrangement. The dried samples, after being examined with X-rays, were used later for extraction studies. For the samples examined moist, rapid drying during exposure to X-rays was prevented by keeping the two ends of a bundle of fibers in contact with a wet blotter. The blotter and fibers were supported by a special holder constructed so that the center of the bundle was opposite a small hole through which the X-ray beam impinged on the sample.

All extractions with organic solvents were made from six to ten hours in a Soxhlet extractor. Preliminary experiments showed chloroform to be the best single extractant. Ethyl alcohol and ether were also used. In heating the fibers with dilute sodium hydroxide or bleaching with sodium hypochlorite, the usual procedures recommended for the preparation of standard cotton cellulose (3, p. 3–7) were followed, with modifications described later. The reagents consisted of 1 per cent hydrochloric acid, 1 per cent sodium hydroxide, and 2 per cent sodium hypochlorite. Heating with sodium hydroxide was carried out in a flask with reflux condenser. Samples treated with sodium hydroxide or sodium hypochlorite were later washed in weak hydrochloric acid. All samples were washed to neutrality with water before drying. The mercerizing alkali solution consisted of 18 per cent sodium hydroxide.

The X-ray diagrams were made with unfiltered copper radiation ($K\alpha = 1.54$ Å) produced in a Philips Metalix tube operating at 28 kilovolts and 25 milliamperes. The X-ray beam was defined by a tube 8 cm. long with openings 0.635 mm. in diameter at each end. The diagrams were registered on a flat photographic film placed 4 cm. from the sample. Samples 1 ± 0.5 mm. thick were exposed 1 to 2 hours. In discussing the X-ray results, "diagram" will be used to refer to the composite diffraction rings obtained from the sample, while "pattern" will refer to the diffraction rings characteristic of a particular component or material of the sample.

RESULTS

The experimental results are described in the chronological order in which they were obtained: (a) untreated fibers as they were removed from the boll, (b) fibers extracted in organic solvents, (c) fibers heated with sodium hydroxide, and (d) fibers purified by extracting with organic solvents, heating with sodium hydroxide, followed by a bleaching with sodium hypochlorite.

UNTREATED FIBERS

Representative X-ray diagrams of fresh samples examined in the dry condition are shown in Figures 1a, 1b, and 1c. The most striking feature of the X-ray diagrams is that there is little or no change until approximately the thirtieth day. Furthermore, up to that time, there is no X-ray evidence of cellulose. Between 30 and 40 days, however, there is a progressive change; the broad, inner, amorphous band of the early diagram (Figs. 1a and 1b) is displaced by the two inner diffraction rings (101 and 10$\bar{1}$) of cellulose, and the outer rings by the most intense (002) ring of the cellulose diagram, which has a slightly larger diameter. At 35 days (Fig. 1c) the cellulose rings may be clearly observed, and at 40 days the characteristic X-ray pattern of mature cotton is obtained.

Similar results were obtained on a series of fresh samples examined in the moist condition. The X-ray pattern, however, is complicated by the superimposed water diagram, and since no evidence of cellulose was obtained until approximately the thirty-eighth day, further samples were not examined.

A third series of preserved samples examined dry gave a clearer diagram and showed X-ray evidence of cellulose at an earlier date than the fresh samples examined under similar conditions. Apparently, these results are due to the preservative (alcohol) acting as an extracting agent, the effect of which will be discussed in the next section.

FIBERS EXTRACTED WITH ORGANIC SOLVENTS

The samples consisted of the fresh material previously examined with X-rays in the untreated dry condition. Figures 1d, 1e, and 1f are representative diagrams after six hours' extraction with chloroform. At the early stages of growth the outer diffraction rings of the untreated diagram are removed. The extracted diagram (Fig. 1d), up to approximately the sixteenth day, consists largely of a broad amorphous band. After 17 or 18 days, the pattern of cellulose can be recognized in the original negative superimposed on the amorphous diagram. This cellulose pattern gradually increases in intensity with age (Fig. 1e) and after approximately 30 days the amorphous diagram disappears and the pattern is that of cellulose (Fig. 1f). A series of preserved greenhouse samples gave similar results.

The outer rings of the early unextracted diagram, which are removed by extraction with chloroform, have spacings of 4.20 and 3.75 Å, which correspond to those of the "primärsubstanz" described by Hess, Trogus, and Wergin (9). The pattern, however, is similar to that of a natural wax, and in further discussions it will be referred to as the "wax pattern." As illustrated in Figure 2e, the waxy material which gives this pattern may be recovered by evaporating the extractant to dryness. Likewise, if mature

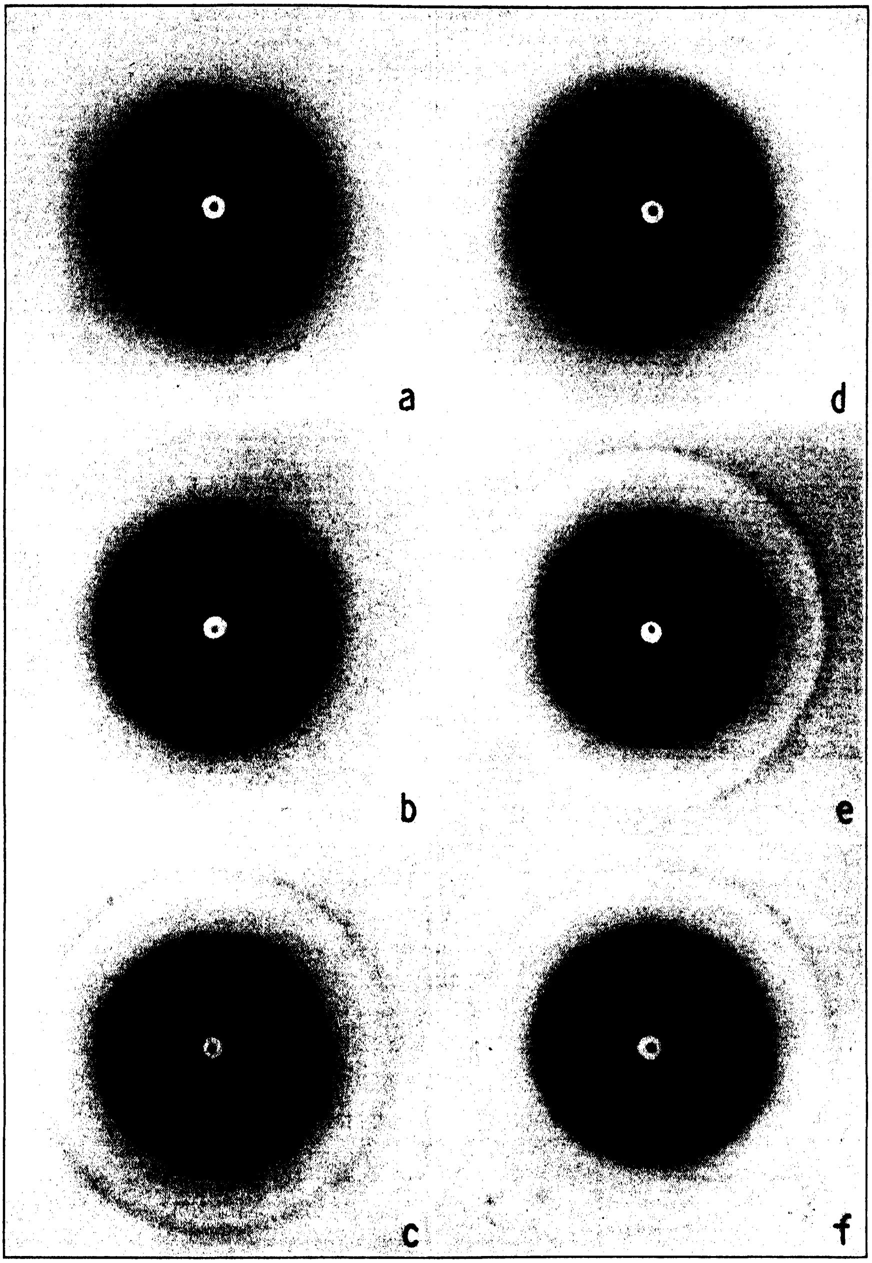


FIGURE 1. Representative X-ray diffraction diagrams of untreated and extracted fibers at various intervals of growth: (a) 10-day, (b) 26-day, and (c) 35-day untreated fibers; (d) 10-day, (e) 26-day, and (f) 35-day fibers extracted in chloroform for 6 hours.

for 10 minutes at room temperature. None of these treatments affected the cellulose X-ray pattern when they were tried on mature cotton. In some of the young fibers below approximately 10 days, the sodium hydroxide and hypochlorite treatments destroyed completely the original outer limiting non-cellulosic membrane, leaving a residue of cellulose particles which was recovered by centrifuging.

FIBERS EXTRACTED WITH CHLOROFORM AND HEATED WITH SODIUM HYDROXIDE

If fibers are extracted with chloroform, heated with sodium hydroxide and bleached with sodium hypochlorite, then both the amorphous and the wax pattern are removed from the diagram, and a cellulose pattern is obtained. This purification treatment was made on two sets of samples; one set was the fresh material previously examined both in the untreated dry and in the extracted condition, while the other was a series of preserved samples. Representative results are shown in Figure 2d, which is the same 23-day-old sample as Figure 2b after extracting with chloroform. The X-ray diagram consists of a crystalline cellulose pattern, as may be seen by comparing it with Figure 1f. A cellulose pattern may also be obtained by the reverse procedure of extracting first with alcohol and ether, followed by heating with sodium hydroxide. As illustrated in Figure 2a, it has been possible by purifying young cotton fibers to obtain a definite crystalline cellulose pattern as early as the fifth day after flowering.

The crystallographic identity of the cellulose identified in the purified fibers to that obtained from mature fibers (7) was further established by changing the native X-ray pattern to a mercerized pattern—a reaction characteristic of crystalline cellulose. The five-day sample shown in Figure 2a was treated with sodium hydroxide of mercerizing strength, washed with one per cent hydrochloric acid and with distilled water, then dried at room temperature. The mercerized X-ray diagram of the dried sample is shown in Figure 2c.

DISCUSSION

The X-ray identification of crystalline cellulose in the young purified fibers is in agreement with the observations of Farr and Eckerson (6) that cellulose particles present in the cytoplasm of the young fibers give microscopic and microchemical reactions characteristic of cellulose if the non-cellulosic material is removed by suitable chemical reagents. Existing evidence indicates that the cellulose detected by X-ray analysis is not formed by a "regeneration," "hydrolysis," "crystallization" or "orientation" of a pre-cellulosic or unorganized substance present in the fiber, but by a "purification" or "unmasking" of crystalline cellulose already present. This is supported by the observation that cellulose particles in the cytoplasm of

the young unextracted fiber remain microscopically intact throughout the purification process, as illustrated in Figures 3a, 3b, and 3c, which are photomicrographs of a 12-day cotton fiber in the untreated, extracted and purified condition, respectively. Furthermore, the waxy and amorphous materials, which are removed by extraction with organic solvents and treatment with sodium hydroxide, can be recovered. These recovered materials give the same X-ray patterns as was removed from the original diagram of the unextracted cotton fiber by the extraction and purification treatments.

The principal reason why crystalline cellulose is not detected by X-ray analysis of the untreated fibers is probably because it is not present in sufficient concentration. Estimations, based on the weight of material recovered from the purified fibers, show fibers below 15 days to have a cellulose concentration in per cent (dry basis) slightly less than the daily age of the fiber (e.g., 10-day fibers have 8 to 10 per cent cellulose). As the fibers mature the ratio of cellulosic to non-cellulosic constituents increases until at maturity the fiber is over 90 per cent cellulose. At the age at which a distinct cellulose pattern is obtained in the untreated fibers, the cellulose has reached a concentration of approximately 30 or 40 per cent. Below that concentration the superimposed patterns of the non-cellulosic materials predominate, and the cellulose must be isolated or concentrated to be detected by X-ray analysis. By way of analogy, waxes are present in the mature cotton fiber, but the percentage is so small there is no evidence of a wax pattern in the mature cotton X-ray diagram. The waxes must be extracted from the cotton and concentrated by evaporating the extractant to dryness before they can be identified by X-ray analysis.

Cellulose concentration alone, however, may not be the only factor prohibiting the X-ray identification of cellulose in the young fibers. If the recovered fractions from the organic solvents and alkali solutions are mechanically mixed with the isolated cellulose in approximately the same proportions as they originally existed in the fiber, the cellulose diagram is not masked as completely as in the original fiber. It is possible that the non-cellulosic materials might be combined with the cellulose so as to influence the nature of the cellulose diffraction pattern, but final interpretations in this connection must await further experimentation in several phases of cellulose analysis.

Because of the irregularities of growth, it is impossible to state the exact age at which cellulose may be detected in the untreated fibers. As shown by the microscopic and microchemical tests of Zakoshchikov, Korzheniovskii, and Ruitikov (13), the rate of development of the cotton fiber, in addition to the species, the soil and weather conditions, depends on the fiber's position on the seed, the position of the seed in the boll, and the position of the boll on the plant. It is probably one or more of these

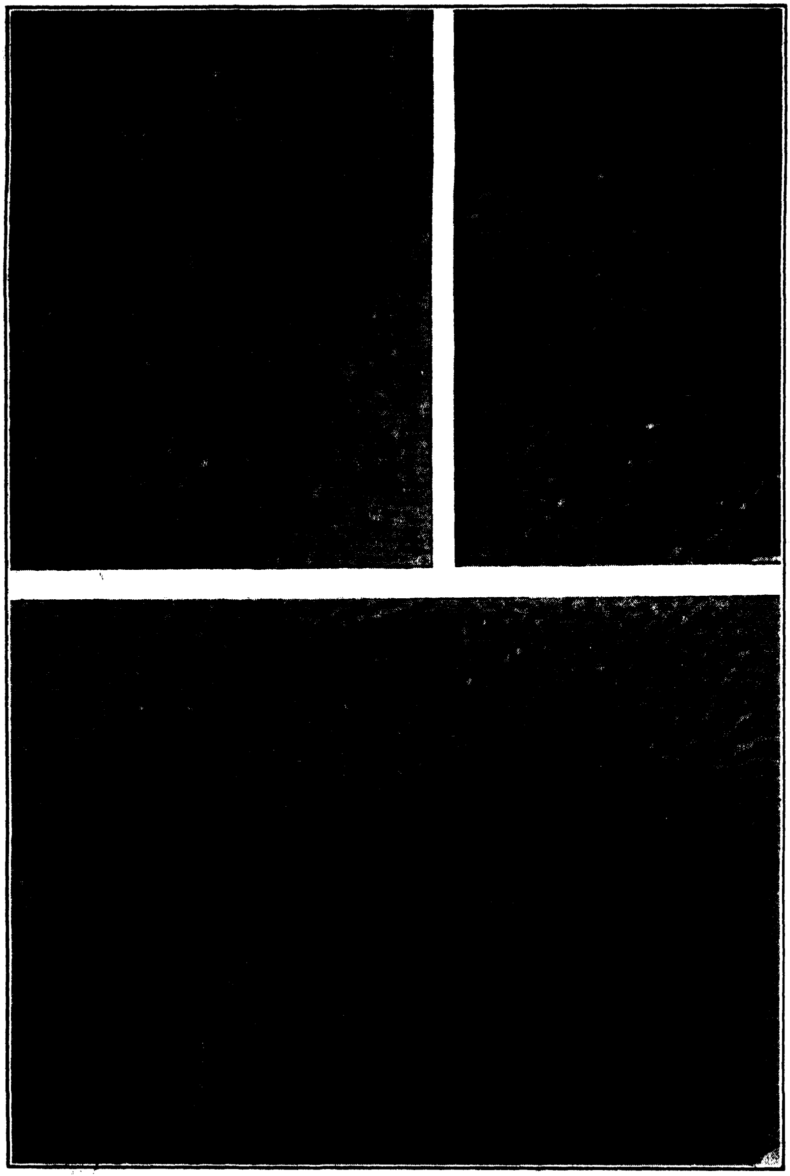


FIGURE 3. Photomicrographs of 12-day-old cotton fiber: (a) median section of untreated fiber, (b) same as (a) after extraction with chloroform, and (c) after further purification by heating with 1 per cent sodium hydroxide and treating with 2 per cent sodium hypochlorite. ×1350.

factors which accounts for the later age at which Hess, Trogus, and Wergin (9) found the untreated fibers to give a cellulose X-ray diagram. The difference between their results and the present results on untreated fibers is well within the range of variation observed in the present investigation. For example, in some untreated fibers the cellulose X-ray diffraction pattern was observed as early as the twenty-fifth day; in others it was absent as late as the fortieth day. The ages given in the present paper represent those that occurred most frequently. These irregularities, however, do not affect the results of the present investigation regarding the presence of crystalline cellulose. The lower limit of five days for the X-ray identification of crystalline cellulose is not because cellulose was not found below that date, but to the experimental difficulties of dissecting large enough samples of the small fibers from which the cellulose can be isolated in sufficient quantities for X-ray examination.

The present data on cotton are in general agreement with X-ray results on other fibers. Ritter and Stillwell (11) found by X-ray examination that certain wood fibers reach maturity in preferred orientation of their cellulose crystallites at the age of approximately 10 days. Heyn (10) found epidermal strips of coleoptiles of *Avena sativa* to have spacings approximately that of pure cellulose. The X-ray results of Hess, Lüdtke, and Rein (8) who found that X-ray diffraction rings of 14-day-old beech shoots were the same as that of a 385-year-old tree, serve to illustrate the stability of crystalline cellulose once it is formed in the living cell.

SUMMARY

1. Cotton fibers ranging in age from 5 to 50 days were subjected to X-ray diffraction analysis. The samples consisted of both fresh and preserved fibers in the untreated condition; after extraction with chloroform; and after further purification with 1 per cent sodium hydroxide and 2 per cent sodium hypochlorite.

2. X-ray analysis showed the presence of crystalline cellulose at approximately 30 to 35 days in the untreated fibers; between 15 to 20 days after extraction; and as early as 5 days in the purified fibers.

3. The cellulose pattern is not present in the X-ray diagram of the untreated fibers because it is masked by a crystalline "wax pattern," which may be removed by extraction with organic solvents, and by an amorphous diagram, which is removed by treatment with dilute alkalies and bleaching.

4. The crystallographic identity of the cellulose obtained from the young purified fibers to that of mature cellulose is shown, not only by its identical unit cell dimensions, but also by the fact that the native X-ray pattern may be changed to a mercerized pattern.

5. The present data corroborate the observations of Farr and Eck-

erson that cellulose is first formed in the cytoplasm as crystalline cellulose particles. Cellulose, after it is once formed, does not undergo a crystalline modification during fiber growth.

LITERATURE CITED

1. CLARK, GEORGE L., WANDA K. FARR, and LUCY W. PICKETT. Analysis of growth and classification of cotton fibers. Indus. & Eng. Chem. **22**: 481. 1930.
2. CLARK, GEORGE L., LUCY W. PICKETT, and WANDA K. FARR. Some practical results of an X-ray analysis of cotton fibers. Science **71**: 293–294. 1930.
3. DORÉE, CHARLES. The methods of cellulose chemistry. 499 pp. Chapman and Hall, Ltd., London. 1933.
4. FARR, WANDA K. Cotton fibers. I. Origin and early stages of elongation. Contrib. Boyce Thompson Inst. **3**: 441–458. 1931.
5. ——— Cotton fibers. IV. Fiber abnormalities and density of the fiber mass within the boll. Contrib. Boyce Thompson Inst. **6**: 471–478. 1934.
6. FARR, WANDA K., and SOPHIA H. ECKERSON. Formation of cellulose membranes by microscopic particles of uniform size in linear arrangement. Contrib. Boyce Thompson Inst. **6**: 189–203. 1934.
7. FARR, WANDA K., and WAYNE A. SISSON. X-ray diffraction patterns of cellulose particles and interpretations of cellulose diffraction data. Contrib. Boyce Thompson Inst. **6**: 315–321. 1934.
8. HESS, KURT, MAX LÜDTKE, und HERBERT REIN. Über Cellulose aus jungen Trieben und altem Kernholz; (V. Mitteilung[1]) über Begleitstoffe der Cellulose.) Justus Liebig's Ann. Chem. **466**: 58–72. 1928.
9. HESS, KURT, CARL TROGUS, und WILHELM WERGIN. Untersuchungen über die Bildung der pflanzlichen Zellwand. Planta **25**: 419–437. 1936.
10. HEYN, A. N. J. X ray investigations of the cellulose in the wall of young epidermis cells. Proc. Akad. Wetensch. Amsterdam, Sec. Sci. **36**: 560–565. 1933.
11. RITTER, GEO. J., and CHARLES W. STILLWELL. Rate of formation of the crystalline structure of wood fibers. Paper Trade Jour. **98**: 37–40. 1934.
12. WERGIN, WILHELM. The growth of plant cell walls. Angew. Chem. **49**: 843–845. 1936. (*Abstr. in* Chem. Abstr. **31**: 733. 1937.)
13. ZAKOSHCHIKOV, A. P., G. A. KORZHENIOVSKII, and M. G. RUITIKOV. A microscopic investigation of cotton fibers at various stages of maturity. Trudui Sredneaziatskogo Nauch.—Issledovatel. Inst. Khlopkovodstvu, Khlopkovoi Prom. Irrigatzii (Trans. Middle Asiatic Sci. Inst. Cotton Culture, Ind. Irrigation) Byull. no. 50: 15–43. 1931. (*Abstr. in* Chem. Abstr. **26**: 5988. 1932.)

OBSERVATIONS ON THE STRUCTURE OF COTTON FIBERS IN THE DARK FIELD

Bruno Rabinowitsch

The microscopic properties of the cellulose particles concerned in the formation of the cellulose membranes of cotton fibers have been described by Farr and Eckerson.[1] Their size (1.5×1.1 microns), their refractive indices (1.565 lengthwise and 1.530 crosswise), their individual reactions to the sulphuric acid—iodine test for cellulose, and their behavior in the living protoplasm during the period of membrane formation are readily discernible by these methods. It is of interest to follow the latter procedure in the fibers of different stages of development by means of the dark-field illuminator and to supplement this previous report with illustrations from the results obtained.

The fiber material was *Gossypium hirsutum* L. (Super Seven, Strain 4) grown in the greenhouse of this Institute. The age of the fibers varied from 7 to 55 days after blossoming time. The young fibers were either isolated and examined in their original state or treated mechanically by cutting and dissecting with a needle. Finally, mature dried fibers of commercial origin were likewise treated mechanically to bring about the separation of the particles in the membranes. A dark-field condenser (Leitz D 1.40) was used in examining and photographing the preparations.

In the young fibers the cellulose particles occur preponderantly as uncombined units. With increasing age of the fiber, chains of particles are formed. It appears, however, that individual particles prevail in the young fibers, whereas the older fibers contain a larger proportion of the chain forms (Fig. 1, A, B, C, D). These observations in dark field corroborate the earlier findings in ordinary and polarized light.

The mature commercial fibers had suffered a considerable amount of membrane disintegration because of their previous chemical treatment (commercial bleach). When treated mechanically, the fibrils and particles were therefore more easily separated than in a mature, untreated fiber. The microscopic mounts obtained showed the reverse of the process of wall formation. All stages of membrane layers disintegrating into fibrils and fibrils, in turn, into cellulose particles were observed. Cut ends of fibers of this type crushed readily and from the funnel-shaped widenings thus produced, separate particles emerged.

[1] Farr, Wanda K., and Sophia H. Eckerson. Formation of cellulose membranes by microscopic particles of uniform size in linear arrangement. Contrib. Boyce Thompson Inst. **6**: 189–203. 1934.

Facilities for these studies were furnished to the author during the time spent as a guest in the Cellulose Laboratory of the Boyce Thompson Institute for Plant Research, Inc.

FIGURE 1. Cotton fibers in dark field. A. Cellulose particles from 7-day fiber (Ocular 20×); B. Strands of cellulose particles in 11-day fiber (Ocular 6×, enlarged $2\frac{1}{4}$ times); C. Cellulose fibrils and particles from 12-day fiber (Ocular 10×); D. Strands of cellulose particles and fibrils in membrane of 55-day fiber (Ocular 10×). (Leitz $\frac{1}{12}$a objective, N. A. 1.32; Leitz dark-field condenser D 1.40.)

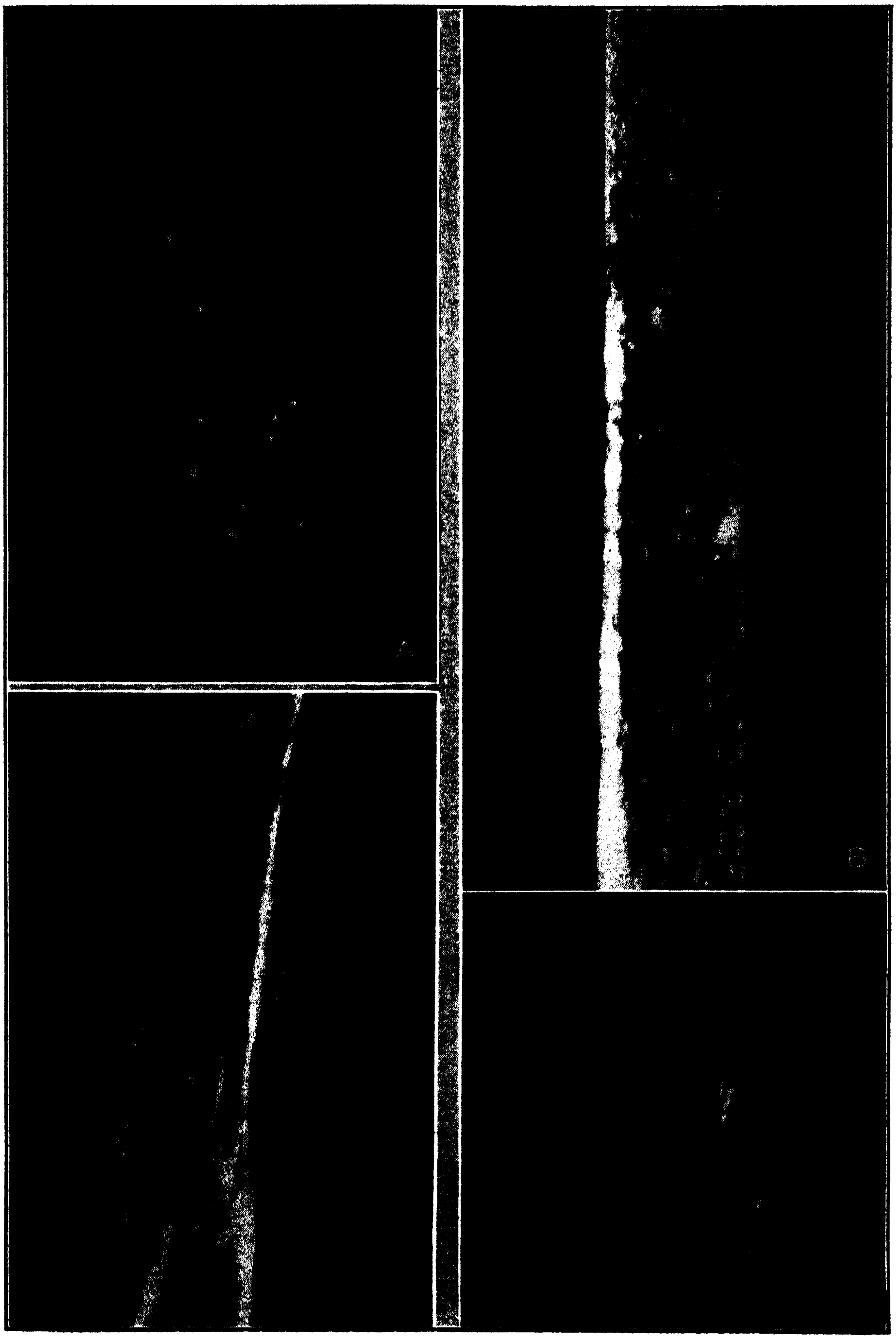


FIGURE 1. For explanation see opposite page.

FORCING FLOWER BUDS IN GARDENIA WITH LOW TEMPERATURE AND LIGHT

JOHN M. ARTHUR AND EDWARD K. HARVILL

Cultural practices which are effective in producing flowers on hothouse gardenia plants (*Gardenia Jasminoides* Ellis) are not well understood. Many growers favor a fairly constant temperature which they claim avoids the dropping of buds. Practically all growers are agreed that it is very difficult to produce flowers on gardenia plants in time for the Christmas market, while there is no difficulty in producing them for the early spring market. Some plants held continuously at high temperatures in greenhouses produce neither large buds nor flowers and growers are often forced back on their last favorite line of defense, holding that it must be the soil. The present study is an attempt to separate the factors which are important first in developing buds and second in forcing them into flower.

METHODS

Early in this study it was observed that very large buds once developed could be opened at any time during the winter by using Mazda lamps for four to six hours each night, while buds on control plants would not open until late in February when natural light had increased in both intensity and daylength. Lamps rated at 500-watts were used in this work as even 300-watt lamps were found to be much less effective. The best arrangement was found to be intermittent light applied all during the night to a total of about six hours. The intermittency was regulated by a thermostat which can be applied in two ways: First, the thermostat can be placed in the center of the house and used to operate a large relay which turns all of the lamps on or off at the same time as more or less heat is needed to keep the house at a certain desirable temperature; or second, a small thermostat of the type used in soil heating cable work can be suspended with its expansion bulb only four or five inches from the tip of the lamp. This type of thermostat usually has a capacity of 25 amperes and can be used to operate two or three lamps directly without a relay. Since the Mazda lamp has 90 per cent of the total energy in the heat or infra-red region and only 10 per cent visible light, the desirability of regulating its heat output by means of a thermostat is at once apparent. This method avoids overheating the plant and the surrounding air and at the same time supplies the needed additional light to give the plant a long day. The insulated greenhouse which is heated and lighted by Mazda lamps (3) is especially satisfactory for growing gardenias. On account of

an almost air-tight construction a high humidity is maintained (80 per cent relative, or above). Both the high humidity and the additional light are important factors in producing an abundance of extra large flowers. In addition the plant responds well to higher carbon dioxide concentrations which can be easily maintained in this type of greenhouse. The temperature during days of brilliant winter sunshine often reaches 95 to 100° F. in the insulated house while during the night the temperature is closely regulated to slightly above 60° F., a range which seems to be very satisfactory for this plant. No doubt even a lower night temperature would be desirable, as will appear in later discussion, if only gardenias were grown. This would, in turn, result in a great saving of electrical energy used in heating and lighting the house. Sodium vapor lamps were used in continuous exposures to open well-developed buds on actively growing gardenia plants. Often only a few days' exposure was necessary. This lamp produces more than twice as much light on the same current consumption as the Mazda lamp. It has a distinct advantage in low cost operation but a disadvantage in high initial cost of lamp equipment and its inherent inability to operate intermittently on relatively long flashes. More detailed study must be made of this light source before it can be recommended without reserve for the work. At present it should be stated that the Mazda lamp is very satisfactory when used as a supplement for sunlight for a few hours each night.

The chief difficulty, as our experience goes in producing flowers on gardenia plants, has been to get flower buds to develop after they are formed. Very small buds will develop on the plants under many conditions of daylength and temperature but only under certain conditions do the buds continue to grow and eventually produce flowers. Figure 1 illustrates three stages of development of the buds. The first stage is reached under many conditions of temperature and daylength. These small initial buds develop into the second stage (Fig. 1 B) only under certain well defined conditions which, if continued, produce the third or full stage of development in approximately two months' time. If the conditions are unfavorable the buds at the first stage may persist for several months or may drop off after a few weeks. When the buds have reached the third stage (Fig. 1 C) during the winter months they can be opened usually in a few days' exposure to additional light each night.

Starting on August 5, a series of tests were made to determine what conditions were necessary to induce buds to develop from the first stage to the second and third. One- and two-year-old plants grown originally from cuttings were used as well as young plants which had been rooted as cuttings seven or eight months previously. A set of plants was placed on a rubber-tired cart which was wheeled out-of-doors at 8 o'clock each morning and left until 4 or 4:30 each evening when it was placed in a

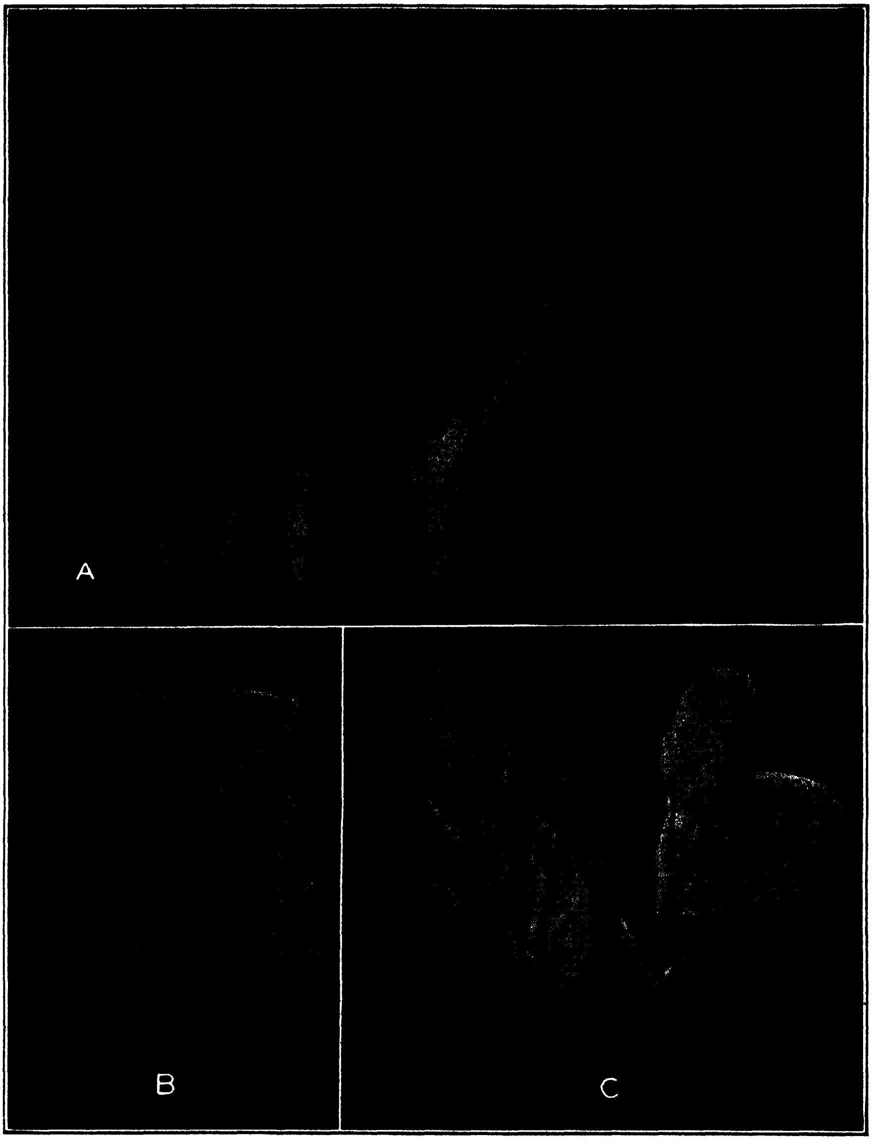


FIGURE 1. Three stages in bud development. A. Small buds which are produced under many conditions of temperature and light. B. Buds develop into this stage only when given a low temperature at night. C. Fully developed buds which can be opened usually in a few days' exposure to artificial light each night.

dark basement. Another set was left in a warm greenhouse (above 70° F.) continuously, while a third set was left out-of-doors continuously. A few plants were grown in the constant light room (1) under continuous sodium vapor light supplemented infrequently with a high pressure mercury vapor lamp. The temperature here was also above 70 ° F. The above conditions were operative until October 15. Only those plants left out-of-doors continuously gave indications of buds developing from stage 1 to stage 2. These plants were exposed each night to the cool night temperatures of late September and October. Neither those plants held in the warm greenhouse nor those of the short-day series which were wheeled into the warm basement each night developed larger flower buds. Those in continuous artificial light grew rapidly but developed no second-stage buds. The plants from the above mentioned tests were all brought inside on October 15. Some were placed in the warm greenhouse (above 70° F.) others were placed in a greenhouse held at 60 to 65° F. Some plants in the cooler house were illuminated each night by 500-watt lamps operated by a thermostat placed in the heat insulated greenhouse, while others were grown in another house at this same temperature (60 to 65° F.) but with no artificial light. All plants in the 60 to 65° F. house developed buds which passed more or less rapidly into stage 2 and later to stage 3. The first flowers opened on December 23 from buds developed on plants brought in from outside on October 15 and given additional light each night. Two of these plants are shown in Figure 2 A. In a short time many flowers appeared in the cool house with additional light. No flowers had opened in the cool house without additional light up to March 5 but many buds had grown to stage 3 and might open at any time. The results in the cool house without light were similar therefore in forcing buds, but the rapidity of their development was greatly decreased because of the low light intensity and short days during the winter months. Plants held continuously in the warm house (above 70° F.) produced no buds at any time which reached the second stage of development by March 5. Many buds of the stage 1 type developed in the warm house, some of which persisted while others dropped off after a few weeks, but none developed further. These effects were independent of the previous treatment—that is, whether the plants had previously received a short day or continuous artificial illumination or the natural daylength of that particular time of year, no buds developed in the warm greenhouse, while buds on all plants continued development at the cooler temperature.

The photograph shown as Figure 2 was taken on January 9. Plant B was grown continuously under sodium vapor light from October to January 9 (temperature above 70° F.); plant C was grown in a warm greenhouse (above 70° F.); and plant D was grown in a cool house (60 to 65° F.) with no additional illumination. Only plant D at the low temperature developed buds. Plants transferred from the warm greenhouse to the cooler

house on January 9 developed flower buds slowly. By March 5, these buds had developed almost to stage 2. It is evident, therefore, that flower buds can be developed at any time during the winter months when plants are transferred from the warm house to a cooler house.

On January 15 another test was made as to whether cool nights and warm days were as effective as continuous low temperature in forcing bud development. A young vigorously growing plant with no buds was

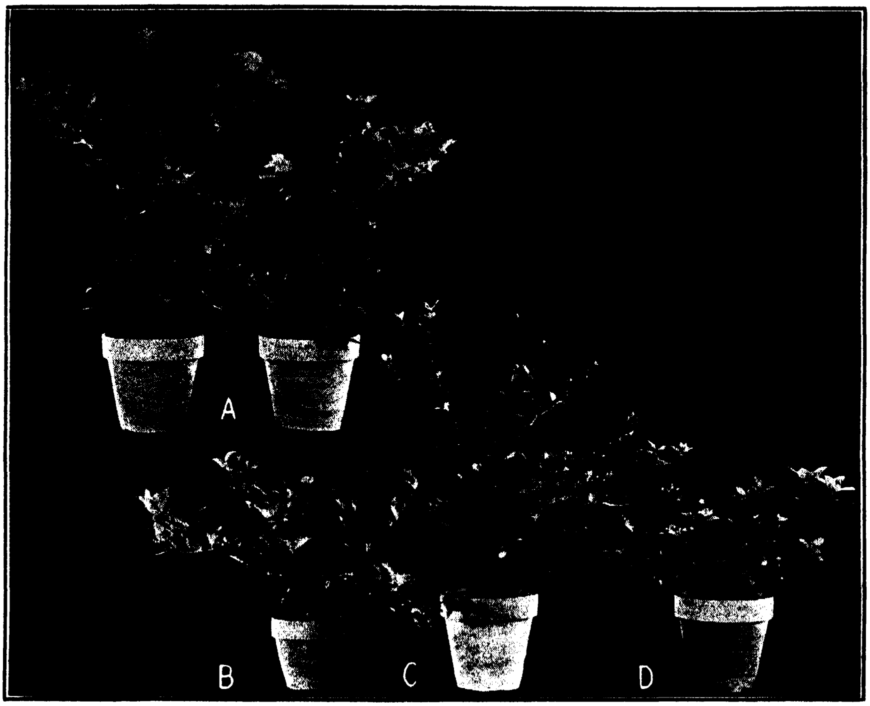


FIGURE 2. Gardenia plants from different temperature and light treatments. A. Flowering in plants brought in from outside on October 15 and given 4 to 6 hours of additional light each night (temperature 60 to 65° F.). B. Grown continuously under sodium vapor lamps (temperature above 70° F.). C. Grown in a warm greenhouse (above 70° F.). D. Temperature same as A, but no additional light. Bud development only in A and D at low temperature, but greatly accelerated in A by additional light.

transferred from the warm greenhouse (above 70° F.) to the constant light room (temperature also above 70° F.) during the day and returned to the cold room (50° F.) each night. It was illuminated from 9 A.M. to 5 P.M. by four sodium vapor lamps. In addition it received two hours' exposure to the 85-watt capillary mercury arc lamp each morning along with the daily exposure to the sodium lamp. This arrangement and the reason for the addition of the capillary arc lamp is discussed in another publication (2). On February 11 many buds were developing rapidly and

by March 1, these buds were half way between stage 2 and 3. The plant is shown in Figure 3 at the right, while the control plant which was kept continuously in a warm greenhouse (above 70° F.) is shown at the left. Both plants are the same age from cuttings taken slightly more than one year ago. From this test it is evident that gardenia plants do not need to be held continuously at low temperatures to develop buds but need only to be exposed to a low temperature at night. This fact is of considerable practical importance to growers as the plants can be kept in a cold room

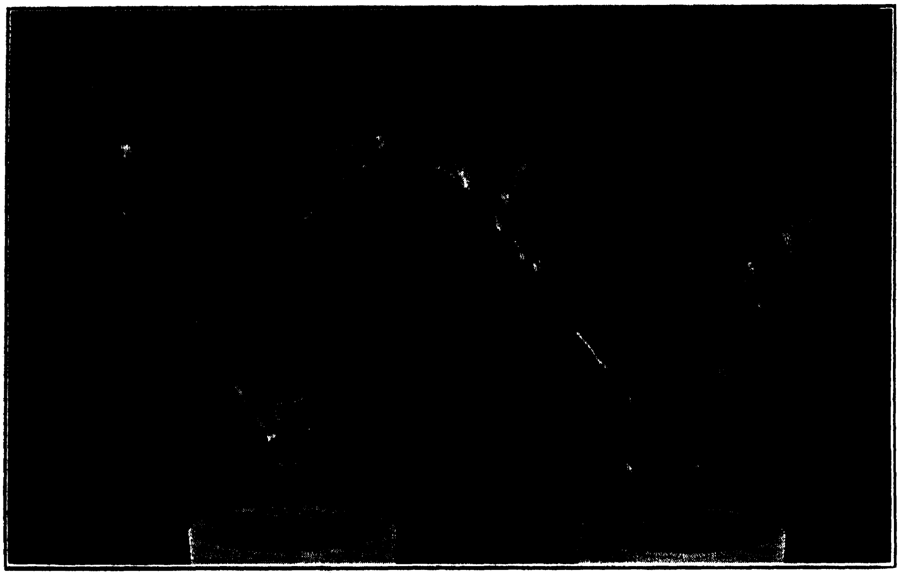


FIGURE 3. Plant at left kept in a warm house (above 70° F.). Plant at right kept in dark at 50° F. each night and under sodium vapor supplemented with 2 hours of capillary mercury vapor each day (temperature above 70° F.).

at night early in the season when the weather is still hot, much more easily than during the day. That is, it would be impractical to attempt artificial cooling of a greenhouse in August or September during the day on account of the high radiation values of sunlight along with the prevailingly high air temperatures, whereas the artificial cooling might be practical at night either in the greenhouse or in an insulated cold room. Further practical tests will be made with this method during the summer. Using this treatment, it should be possible to produce flowers in November and December as well as in February or March if the cold treatments are started sufficiently early.

Cuttings of the Hadley variety were received from a grower on November 12. These were treated with root-inducing substances by A. E. Hitchcock, using methods published for other varieties (5). The plants from which the cuttings were taken had been held at approximately 52° F.

The young rooted plants were potted separately in January and some of these were placed in the high temperature house (above 70° F.) while others were kept in the low temperature house (60 to 65° F.). Many of the young plants held at low temperature had buds approaching stage 2 by March 5, while none of the plants had produced buds developing beyond stage 1 in the high temperature house. It is apparent therefore, that low temperatures are effective in developing buds even on young plants while higher temperatures inhibit bud development. Rooted cuttings so far have grown more rapidly at the higher temperature so that this appears more desirable for producing large young plants quickly.

DISCUSSION

From this series of tests it is evident that flower bud development in gardenia depends upon cool night temperatures below 65° F. Many growers prefer temperatures much lower than this, often as low as 50 to 55° F. Day temperatures do not appear so important as they often ran above 95° F. in the insulated greenhouse on bright days during this study, yet this house seemed ideal for gardenias. High humidity and increased carbon dioxide supply, however, are also factors which favor gardenia development in the insulated house. Bailey (4, v. 2, p. 1315) states that the night temperature for gardenias should not go below 65° F. The true statement should be that the night temperature should never go *above* 65° F. if flower buds are to develop. Neither short days nor long days are effective in producing bud development unless the plants receive cool night temperatures. When given cool nights both bud development and flower production are greatly accelerated by long days. Well-grown buds can be opened usually in a few days' exposure to additional light each night using Mazda lamps or by continuous exposure to sodium vapor lamps. Mazda lamps rated at 500 watts were found to be much more effective than 300 watts for aiding in bud development and in opening buds. When the lamps are operated by thermostats so that both heat and light are applied intermittently all danger of overheating the plants is avoided. Two methods of operating lamps by means of thermostats were discussed in the text. Probably the soil-heating thermostat suspended directly beneath a lamp would be most practical for forcing only a few plants which can be illuminated by two or three lamps while the thermostat and power relay would be more suitable for large installations where many lamps are operated simultaneously.

The gardenia is not alone in its low temperature requirements for flower development. Thompson (8) found that a temperature above 70° F. prevented flower and seed stalk development in celery while temperatures of 60° F. favored such development during the first year of growth from seed. Miller (6) found a similar situation in cabbage plants where cool temperatures (below 60° F.) forced flower development. Other biennials have since been added to this list. Flower bud development in many

bulbous plants, such as hyacinth and daffodil (7) are known to be favored by low temperatures. Winter wheat is an example of a common crop plant which requires low temperature for flower stalk production. There is an ever increasing list of plants, widely separated botanically, which are attuned to a low temperature period during some portion of their growth. This fact indicates that such plants are all natives of temperate climates where cool temperatures prevail for a definite period each year.

The gardenia both on account of its beautiful fragrant flowers and its fine glossy leaves is a highly desirable greenhouse plant and no doubt will be grown much more extensively once its cultural peculiarities are well understood. Many growers have been discouraged in the past when whole collections of such plants, having received every known care in a high temperature house, such as mixed fertilizers, bottom heat and good ventilation either failed entirely to flower or at best flowered only sporadically.

SUMMARY

1. Gardenia plants with large well-developed flower buds can be brought into flower during the winter months by means of additional light from 500-watt lamps used intermittently for a peroid of four to six hours each night.

2. A cool temperature is required during early stages of bud development as plants held at a high temperature (above 70° F.) failed to develop buds. Temperatures of 60° F. or less are favorable to bud development.

3. Low temperatures need to be applied only at night for bud development, while temperatures during the day of 95 to 100° F. (due to high radiation intensities) are not unfavorable.

4. The insulated greenhouse is especially favorable for gardenias on account of the high humidity, high carbon dioxide, intermittent light at night, and well controlled temperature.

LITERATURE CITED

1. ARTHUR, JOHN M., JOHN D. GUTHRIE, and JOHN M. NEWELL. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930. (*Also in* Contrib. Boyce Thompson Inst. **2**: 445–511. 1930.)
2. ARTHUR, JOHN M., and EDWARD K. HARVILL. Plant growth under continuous illumination from sodium vapor lamps supplemented by mercury arc lamps. Contrib. Boyce Thompson Inst. **8**: 433–443. 1937.
3. ARTHUR, JOHN M., and L. C. PORTER. A new type of insulated greenhouse heated and lighted by Mazda lamps. Contrib. Boyce Thompson Inst. **7**: 131–146. 1935.
4. BAILEY, L. H. The standard cyclopedia of horticulture. 3 v. Macmillan Co., New York. 1935.
5. HITCHCOCK, A. E., and P. W. ZIMMERMAN. Effect of growth substances on rooting response of cuttings. Contrib. Boyce Thompson Inst. **8**: 63–79. 1936.
6. MILLER, JULIAN C. A study of some factors affecting seed-stalk development in cabbage. New York Agric. Exp. Sta. [Cornell] Bull. 488. 46 pp. 1929.
7. SLOGTEREN, E. VAN. The early-forcing of daffodils. Inst. voor Phytopath. Lab. voor Bloembollenonderz. Lisse 47. 1933. (*Also in* R.H.S. Daffodil year-book. **1933**: 41–49.)
8. THOMPSON, H. C. Premature seeding of celery. New York Agric. Exp. Sta. [Cornell] Bull. 480. 50 pp. 1929.

RELATION OF STANLEY'S CRYSTALLINE TOBACCO-VIRUS PROTEIN TO INTRACELLULAR CRYSTALLINE DEPOSITS

HELEN PURDY BEALE[1]

INTRODUCTION

About two years ago, Stanley (25) reported the isolation of a crystalline protein from the expressed juice of tobacco plants affected with ordinary tobacco-mosaic virus. The crystalline protein was described as possessing the properties of tobacco-mosaic virus, among them the ability to induce mosaic disease in susceptible plants. Stanley (26) has subsequently published various improvements in his method of isolation but the essential steps in the procedure are as follows. The shoots of mosaic-diseased plants are harvested and frozen, then ground in a food chopper while frozen. The juice is extracted with a hand press and the globulin fraction is precipitated by 0.4 saturation with ammonium sulphate. The globulin fraction is further freed from accompanying substances by adsorption on celite at pH 4.5 and subsequent elution with water adjusted to pH 8.0. Needle crystals, about 0.02 to 0.03 mm. in length, are then precipitated from the purified concentrate by the addition of a small amount of ammonium or magnesium sulphate and acidification with acetic or hydrochloric acid. A complete account of Stanley's work on tobacco-mosaic-virus protein with an appended bibliography has just been published (29). Stanley's preparation of needle crystals from expressed juice has been confirmed in England by Bawden, Pirie, Bernal & Fankuchen (1), and in the United States by Takahashi & Rawlins (31), as well as in this laboratory by the author.

ISOLATION OF STANLEY'S CRYSTALLINE TOBACCO-VIRUS PROTEIN

From virus extracts of various hosts. The author used as sources of virus extract *Nicotiana tabacum* L. var. Turkish, affected separately with ordinary tobacco-mosaic virus and the attenuated strain described by Holmes (15) in which a masking of mottling symptoms occurs. Crystalline material was also isolated from mosaic-diseased Turkish tobacco, which had been dried at room temperature, previous to extraction. *N. tabacum* L. var. White Burley, affected with the ordinary tobacco-mosaic virus, also yielded needle crystals upon concentration and purification of the

[1] Assistant plant pathologist, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, and research associate in bacteriology, College of Physicians and Surgeons, Columbia University, New York, New York.

expressed juice. *Solanum nigrum* L. var. *nodiflorum* affected with the aucuba strain of tobacco virus, and *Petunia* sp. affected with ordinary tobacco virus were likewise used as sources of virus extract for the production of needle crystals. In the latter case an excellent yield was obtained from as small a volume as 23 cc. of virus extract.

From a mixture of two viruses. The crystalline protein was isolated from Turkish tobacco affected with streak disease which is caused by a combination of ordinary tobacco virus (Johnson's tobacco virus 1) and the potato X virus (Johnson's tobacco virus 5). In the production of streak disease the two viruses were multiplied simultaneously in the same plants. The crystalline material thus obtained appeared the same as that isolated from plants infected with tobacco virus only, but it was necessary to test for the presence of a similar potato X protein. Solutions of the streak protein were therefore inoculated on *Nicotiana glutinosa* L. and after a suitable incubation period local lesions characteristic of tobacco virus appeared but the plants failed to develop the systemic infection typical of potato X virus on this host. Evidently the protein possessed the property of inducing tobacco-mosaic disease and not potato X mosaic but this did not exclude the possibility that the potato X virus had become inactivated during the preparation of the protein from streak extract and was present in the inactive form. In view of the fact that there is such a close correlation between the antigenic and virus content of extracts of both of these viruses and since antigenic substances can be demonstrated in the virus extract of plants after partial (21) or complete (27) inactivation of virus, the serum-precipitin reaction was employed to detect the possible presence of precipitin specific for potato X-virus extract. Solutions of crystalline protein isolated from streak extract were tested with antiserum to potato X-virus extract for the presence of preciptin but the results were negative although precipitin specific for tobacco-virus extract was demonstrable in high dilutions of the protein. It would appear that precipitinogen in streak extract specific for potato X virus has either been separated from that of the tobacco virus during the preparation of crystalline tobacco-mosaic protein or that the less stable potato X virus in losing its activity has lost its antigenicity as well. Another possibility which should be taken into consideration is the possible alteration in the specificity of tobacco and potato X antigens, as the result of simultaneous multiplication of the two viruses in the same host. This matter was investigated in the summer of 1935, in a series of experiments undertaken in cooperation with Mr. D. K. O'Leary.

SERUM TESTS FOR ANTIGEN SPECIFICITY IN MIXTURES OF VIRUSES

At this time antiserums specific for potato X and ordinary tobacco virus, multiplied in tomato plants, were produced separately in rabbits

according to the method described earlier (22). A third antiserum was obtained by injection of rabbits with extract from tomato plants in which the two viruses had been multiplied simultaneously. Cross titrations were made to determine the presence or absence of specific precipitin to the two virus extracts in the three different antiserums. Eventually precipitin-absorption tests were resorted to (22) and it was found that precipitin specific for both tobacco-virus antigen and potato X antigen were present in antiserum to streak extract. Furthermore, the precipitin could be removed independently by absorption with the homologous antigen from plants in which the two viruses had been multiplied separately. From these results it was concluded that the tobacco virus and potato X virus retain their antigenic identities whether multiplied in separate plants or in the same plant simultaneously. Consequently, the failure of solutions of the crystalline tobacco-virus protein, prepared from streak extract, to give positive precipitin reactions with antiserum to potato X virus, can not be attributed to an alteration in antigenic specificity.

Several attempts to crystallize a protein from Turkish tobacco affected solely with potato X virus, following the procedure so successful in the case of tobacco-mosaic virus, yielded negative results. Apparently no appreciable potato X antigen is present in the solution of tobacco-virus protein.

EXAMINATION OF LIVING DISEASED CELLS FOR SOURCE OF STANLEY'S CRYSTALS

Following Stanley's announcement that a crystallizable protein possessing the properties of tobacco-mosaic virus was present in virus extract in an estimated concentration of approximately 1 part in 500 (26), it seemed logical to turn back to the diseased plant tissue in an attempt to identify the protein with some of the earlier recognized cell inclusions characteristically associated with tobacco mosaic and other virus diseases of plants, higher animals, insects, fish, and birds.

Cell inclusions were observed and described in pathologic tissues, obtained from diseases since classified as filterable viruses, many years prior to the recognition of filterable viruses as etiologic agents of disease. Following proof that an infection could be induced by inoculation of the filtrate obtained by passing extracts from diseased tissues through filters known to withhold ordinary bacteria, various attempts were made to identify cell inclusions as the infectious principle. Iwanowski in 1903 made a careful cytologic study of tobacco affected with mosaic disease (17). While he ultimately concluded that the malady was due to small rod-shaped bacteria, which he represents as zoogloeal masses inside the cell, he also figured the amoeboid bodies present in the cytoplasm of the plant. These plasma-like vacuolate inclusions, called "X-bodies" by Goldstein

(10) have analogues in many of the filterable virus diseases and have been variously described as representing a stage in the life cycle of an organism, an aggregate of microorganisms, a combination of virus and substances derived from the host cell, nuclear fragments or degenerative products

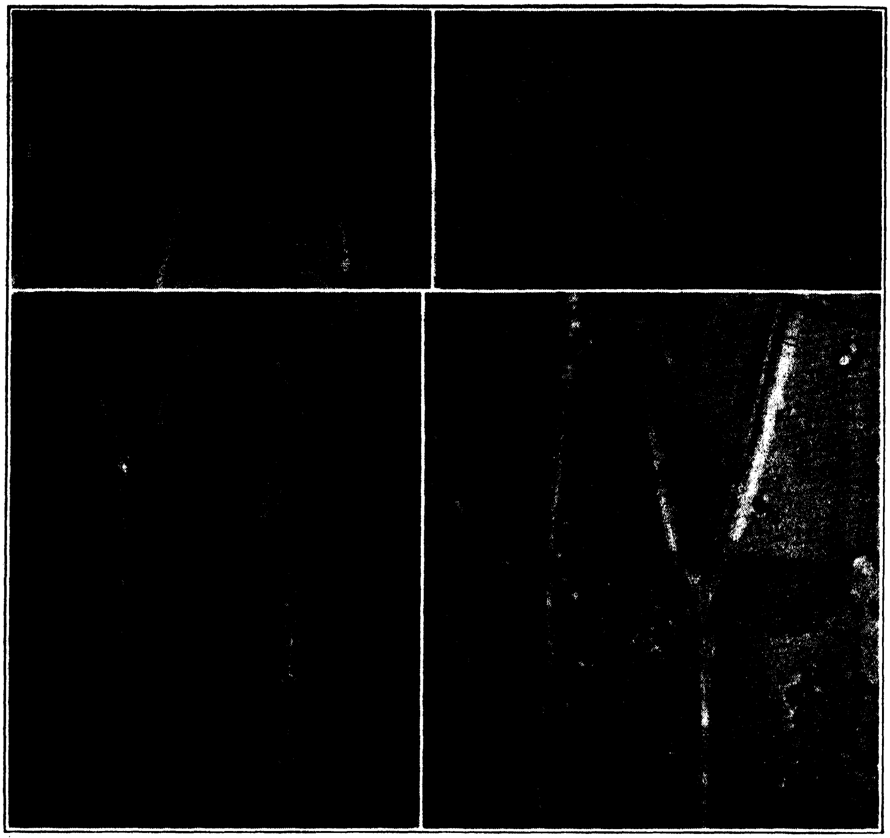


FIGURE 1. (A) Trichome of living mosaic-diseased Turkish tobacco containing X-body with dark granules. ×600. (B) Same cell with crystalline deposits in focus. ×600. (C) Epidermal tissue of living healthy Turkish tobacco. ×690. (D) Epidermal mosaic-diseased tissue showing large deposits of crystalline material characteristically present in cells from chlorotic areas. ×690.

arising from the reaction of the host to virus. An example of this type of cytoplasmic inclusion may be found in Figure 1 A. The X-body, embedded in the cytoplasm of a tobacco trichome, was photographed in the living cell. Dark granules were present in many of the vacuoles of the X-body.

Figure 1 B is a microphotograph of the same cell at a different focus. The X-body is blurred in appearance but a second type of intracellular

inclusion, equally characteristic of tobacco-mosaic disease, has been brought into focus, viz., deposits of a crystalline material which is doubly refractive under the polarized microscope. Two groups of crystals are visible below and one just above the X-body. Iwanowski (17) referred to this material as colorless, crystal-shaped, deposits of wax-like material, but having a lower refractive index than wax. He further described thin plates of a similar material, which Goldstein (10, 11) later explained was one and the same substance, occurring as plates in basal plane and oblong in side view. Iwanowski noted the development of cross striations in the crystalline material found in prepared sections, which he attributed to the influence of acids in the fixatives. Goldstein confirmed this view by mounting strips of mosaic-diseased epidermal tissue in water and adding drops of Flemming's solution or chromo-acetic acid to these fresh preparations. After standing overnight striated masses, stained yellow by the fixative, were found in place of the typical crystal forms. Goldstein expressed the opinion that faint striations were present in the crystalline material before the addition of fixatives but that acid intensified these striations. In a later paper Goldstein (11, p. 566) made the following statement "Upon the addition of Flemming's solutions, as Iwanowski (1903) also noted, the crystals show this striation more distinctly, and often appear to be made up of distinct rods or needle crystals arranged side by side." This observation was accurate in the light of our present findings. Among others Rawlins & Johnson (23), Hoggan (13), and Smith (24) have made cytologic studies of tobacco-mosaic disease and all have described and figured the crystalline striate material. Prior to Grant (12), the crystalline substance had been reported in only one plant outside of the SOLANACEAE, namely *Martynia louisiana* Mill. (14), a member of a closely related family. It was thought for a great many years that tobacco virus was very limited in host range. Probably due to this belief, the crystalline material was generally regarded as peculiarly related to solanaceous hosts. Grant (12) has since observed striate material and X-bodies in several of the 29 non-solanaceous plants, to which he has successfully transmitted tobacco virus. The 29 hosts showing positive infection, representing 14 widely separated families, comprise about 24 per cent of the total 121 species tested for susceptibility. Clinch (8) reported occasional striated bodies in one of the potato-mosaic diseases, very similar to those associated with tobacco mosaic. No inoculations were made to tobacco so the possibility of contamination can not be eliminated. The striated material appears to be specific for strains of tobacco-mosaic virus.

The question of the relation of the infectious principle to the mosaic pattern was a matter of great interest among the early investigators. Some claimed that the diseased portion of the leaf was localized chiefly in the green areas, while others maintained that the chlorotic areas rep-

resented the infectious portions of the leaf. The majority seemed to favor the latter opinion, a view that has received general support. In Figure 6 E, the leaf removed from a mosaic-diseased plant shows the characteristic mosaic pattern, comprised of alternately dark-green and chlorotic areas. The virus is chiefly concentrated in the chlorotic regions, which exhibit strikingly abnormal histologic pictures when the leaf becomes infected before it is fully developed. A cross section through such a mosaic-diseased leaf typically reveals a double layer of brick-shaped palisade cells in the dark-green areas, while the thinner chlorotic regions exhibit a single layer of cuboidal, or isodiametric cells beneath the epidermal layer, which are scarcely distinguishable from the spongy parenchyma. The intercellular spaces are notably reduced. The chloroplasts are abnormal in size and reduced in number in the chlorotic areas, while the chlorophyll is markedly paler. As Goldstein emphasized, the histologic abnormalities are not so pronounced when infection occurs after the leaf is fully developed.

While X-bodies and striate material have been reported by Goldstein throughout the leaf, as well as in the stem, root, and flowers, some of the best material for cytologic study is obtainable by stripping bits of epidermal tissue off the back of the midrib of a leaf, a petiole, or the stem within a short distance from the growing tip. In prepared sections, the intracellular inclusions are concentrated in the chlorotic areas, predominating in the trichomes and epidermal layers.

The material used in the studies described in this paper were confined to strips of epidermis from the back of the midrib of mosaic-diseased leaves as described in a preliminary report (3). These bits of tissue were mounted in water and observed in the living condition without the use of fixatives or stains. Microphotographs taken during the examination of the diseased tissues are reproduced in this article.

Figure 1 C represents healthy tobacco tissue, and Figure 1 D, mosaic-diseased cells. It will be noted that large deposits of crystalline material present in the latter, are absent in the healthy cells. Figure 2 illustrates the great variety in outline which the crystals may present. A good example of twinning may be seen in the lower right hand corner of Figure 2 A. In the upper right hand corner of the same section, a crystal which is probably irregular in outline appears oblong in shape due to the fact that it is observed in side view. The crystals in Figure 2 B show both face and side view.

Figure 3 A shows a microphotograph of tobacco trichomes containing hexagonal plates of the crystalline material. The two lower hairs show oblong crystals, which are plate crystals in side view. A small ovoid X-body is visible at the right of the hexagonal crystal in the uppermost tobacco trichome. A large deposit of crystalline material, irregular in outline, is to be found in the petunia cell in Figure 3 B. Three hexagonal plates

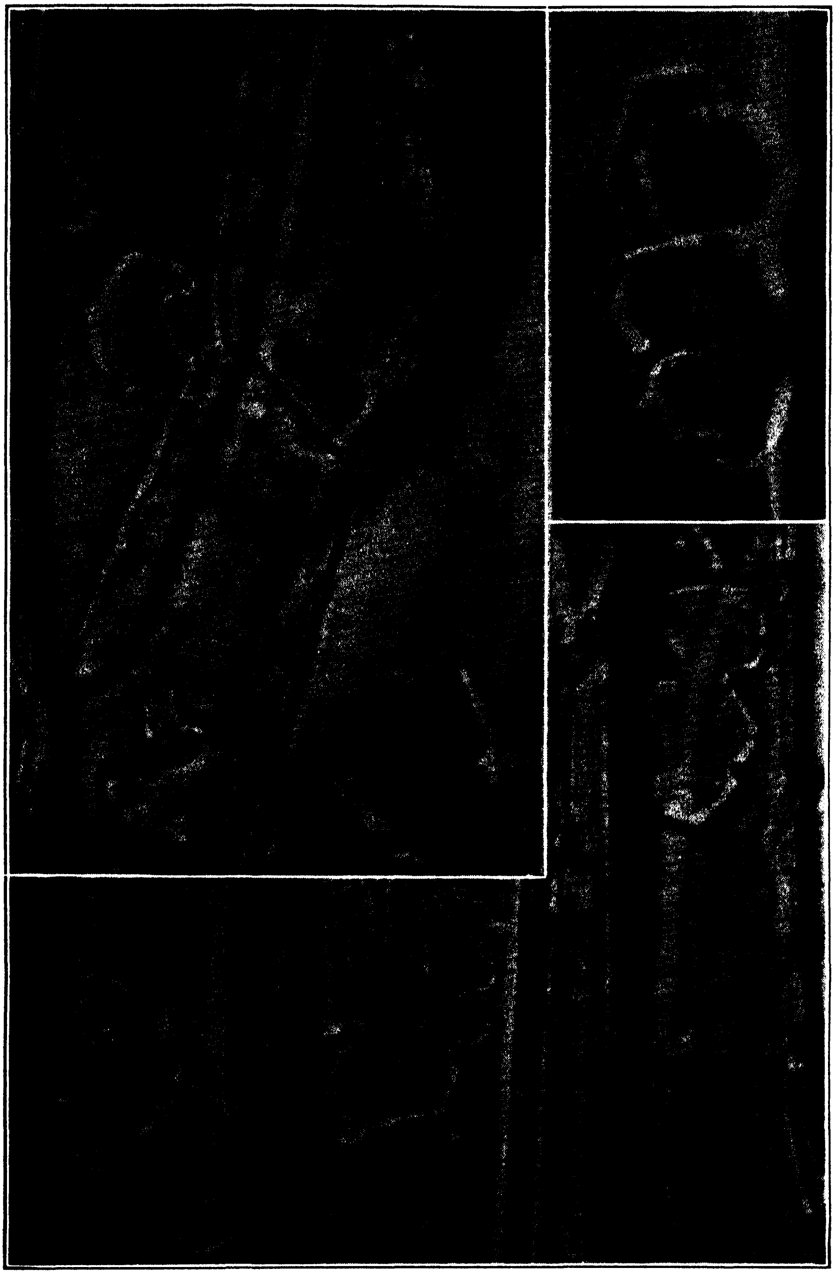


FIGURE 2. Epidermal cells and trichome of living mosaic-diseased Turkish tobacco containing plate crystals. (A) An example of twinning in the lower right hand cell and side view of a plate crystal in the upper center cell. (B) Both face and side view of two crystals. (C) Showing great irregularity in outline of crystals. ×950.

are also present in the epidermal cell of petunia in Figure 3 C. It is interesting to note that from a study of the X-ray diffraction pattern of crystalline tobacco-virus protein prepared from virus extract, according to the general method of Stanley, by Bawden, Pirie, Bernal & Fankuchen (1, p. 1052), the authors conclude that "The dry gel gives the most definite pattern . . . showing five lines . . . corresponding to the first five possible reflections of a hexagonal close-packing with intermolecular distance of 152.0 ± 0.5 A. The wet gel gives three distinct lines which seem to correspond to hexagonal close-packing with intermolecular distance of 210 A., but this varies slightly with the composition." In the light of these findings, it would not be incompatible to predict that the crystalline material might assume a visible hexagonal form. Apparently this is the case.

Figure 3 D demonstrates the shift in the position of the crystals due to the streaming of the cytoplasm in the host cell. Photographs were taken at intervals of a few minutes and the crystal has turned over so that the lower face in the first picture becomes visible in the last. In the third view, the reflection of light from the highly refractive crystal in the adjacent cell is evident. Figure 3 E and F also show a change in position of the three crystals, occurring within a few minutes. The host in each case is petunia, an epidermal cell in Figure 3 D and a trichome in Figure 3 E and F.

After becoming familiar with crystalline deposits in the living diseased cell mounted in water, dilute acid of approximately pH 1.3 was added at the side of the cover slip and drawn under with a strip of filter paper held at the opposite side of the cover slip. The effect observed was recorded in the following microphotographs. Figure 4 A shows an epidermal cell of tobacco which contained two plates in side view. Upon the addition of the acid, the oblong crystals swelled and developed cross striations. In the tobacco trichome shown in Figure 4 B, the individual needle crystals have formed and a number of them have slipped out of the compact mass and are seen free in various parts of the cell. During the growth of the initial small needle crystals, the entire mass seemed to spread out like a fan just prior to the breaking loose of the individual needles from the aggregate.

When the crystals are observed in full face view, as in the tobacco trichome in Figure 4 C, the parallel striations developing upon addition of acid at right angles to the face of the crystals are not visible. Instead, since one is looking down upon the ends of the needle crystals, the addition of acid produces a speckled appearance, as shown in Figure 4 D. Cracks frequently develop, and the crystalline mass in face view seems to swell at first and then shrink. With the correct focus, cross striations can often be detected when the crystal is slightly tilted so that a side is partially visible. In connection with the vastly different appearance of a crystal under the influence of dilute acid, depending upon whether the crystal is

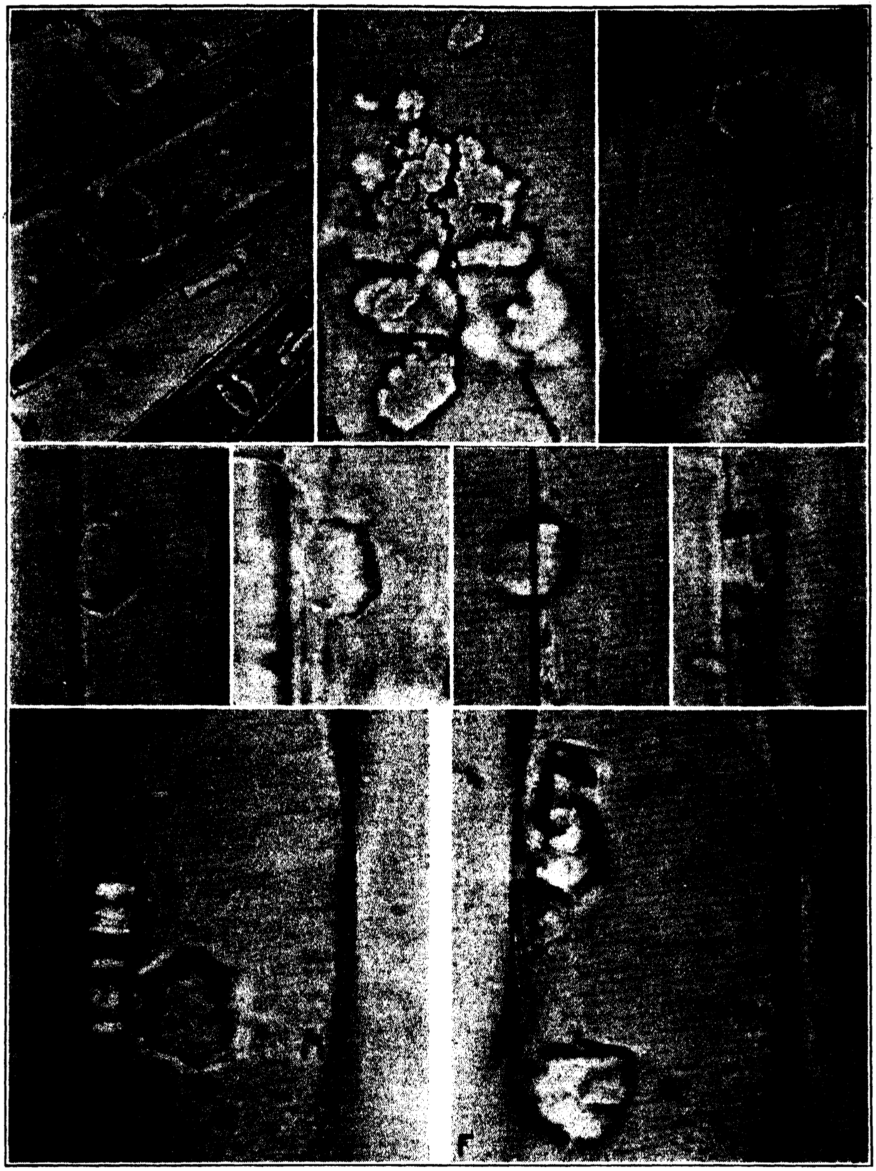


FIGURE 3. (A) Living trichomes of mosaic-diseased Turkish tobacco containing plate crystals, hexagonal in face view, oblong in side view. X-body at right of crystal in upper trichome. ×560. (B) Crystalline deposits in mosaic-diseased petunia showing irregularity in outline. (C) Hexagonal plate crystals in epidermal cell of petunia. (D) Living epidermal cell of mosaic-diseased petunia. Same crystal photographed at intervals of a few minutes. Note shift in position due to streaming of host cytoplasm. (E, F) Living trichome of mosaic-diseased petunia containing three crystals photographed at an interval of a few minutes. Note how the streaming of host cytoplasm has shifted their position. B–F ×640.

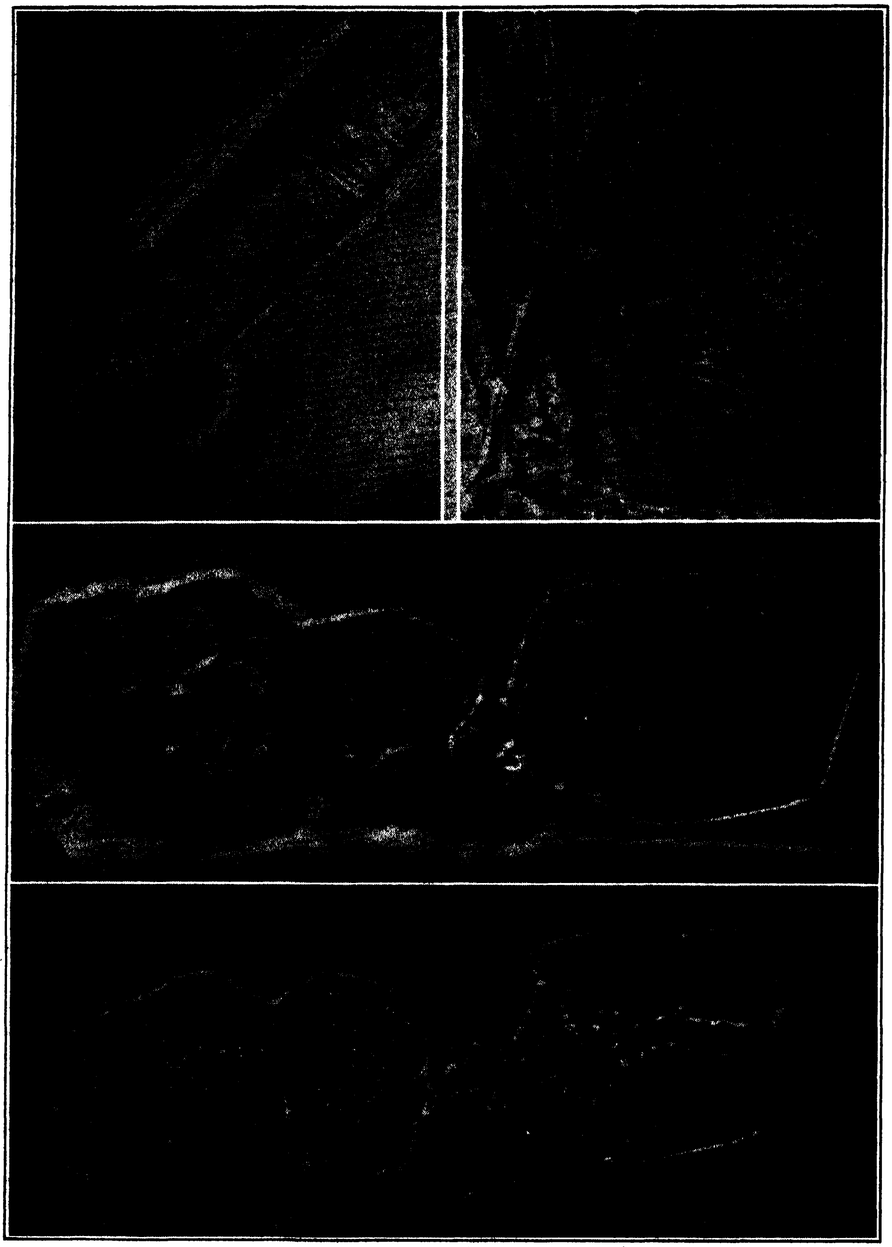


FIGURE 4. (A) Epidermal cell of mosaic-diseased Turkish tobacco to which dilute HCl has been added. Note striations in oblong crystalline mass. (B) Striated mass from which individual crystals have slipped out. (C) Living trichome of mosaic-diseased Turkish tobacco containing a large crystalline deposit. (D) Same cell after addition of dilute HCl. Note crack in crystal at right and speckled appearance of faces due to end view of needles. ×920.

viewed in the basal or lateral plane, Iwanowski's early observations are of considerable interest. In his fixed preparations he describes zoogloea-like masses of very short rod-shaped bacteria which take Loeffler's methylene-blue stain after heating for one to two minutes. He records the disappearance in prepared sections of the smooth crystalline plates found in the living material, and states that the blue zoogloeal masses in the fixed tissue seem to correspond to them. He feels that no confirmation of his view is possible due to his failure to stain the plates in the fresh tissue. It seems probable that Iwanowski was describing the effect of acid on the crystals in face view as zoogloeal masses, and that the stain had freely penetrated the disorganized crystalline mass.

Figure 5 A is a strip of epidermal tissue, from the midrib of a petunia leaf. One of the cells contains a cluster of crystals, some in face view and one crystal turned on the side. Acid was added and a speckling has developed on the faces of the crystals and cross striations along the edges. Figure 5 B is epidermal tobacco tissue showing crystalline masses, unaffected as yet by the acid, and a cell entirely filled with needles at the base of the trichome. The rate of the reaction differs in different cells, depending upon the penetration of the acid and the thickness of the mass of crystalline material. Thin individual crystals often go to pieces without forming clearly defined needles, whereas if less acid is added needle-shaped crystals will gradually form.

Figure 5 C shows mosaic-diseased epidermal cells of tobacco representing a later stage in the transformation of the crystalline mass to needles. Figure 5 E is a similarly treated glandular hair, while Figure 5 D is a microphotograph of a suspension of needle crystals prepared from virus extract according to the method of Stanley and is presented for comparison.

Figure 6 A illustrates the needle crystals in a trichome of petunia. The host cell is in general larger than that of tobacco and the crystals seem to be larger. Figure 6 B shows epidermal cells of tobacco filled with needle crystals.

Needle crystals have been successfully produced in all of the hosts tested up to the present time. Intracellular precipitation of the needles has been accomplished in tobacco of two varieties, Turkish and White Burley, petunia, tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum* sp.), and *Solanum nigrum* L. var. *nodiflorum*. Dilute sulphuric, hydrochloric, acetic, and nitric acids have been added to lower the pH of the cell and induce the transformation to the needle form of crystals. In several cases, no acid was added, but a few drops of saturated magnesium sulphate solution were run under the cover slip with equally good results.

Acid should be added cautiously and if the reaction proceeds too

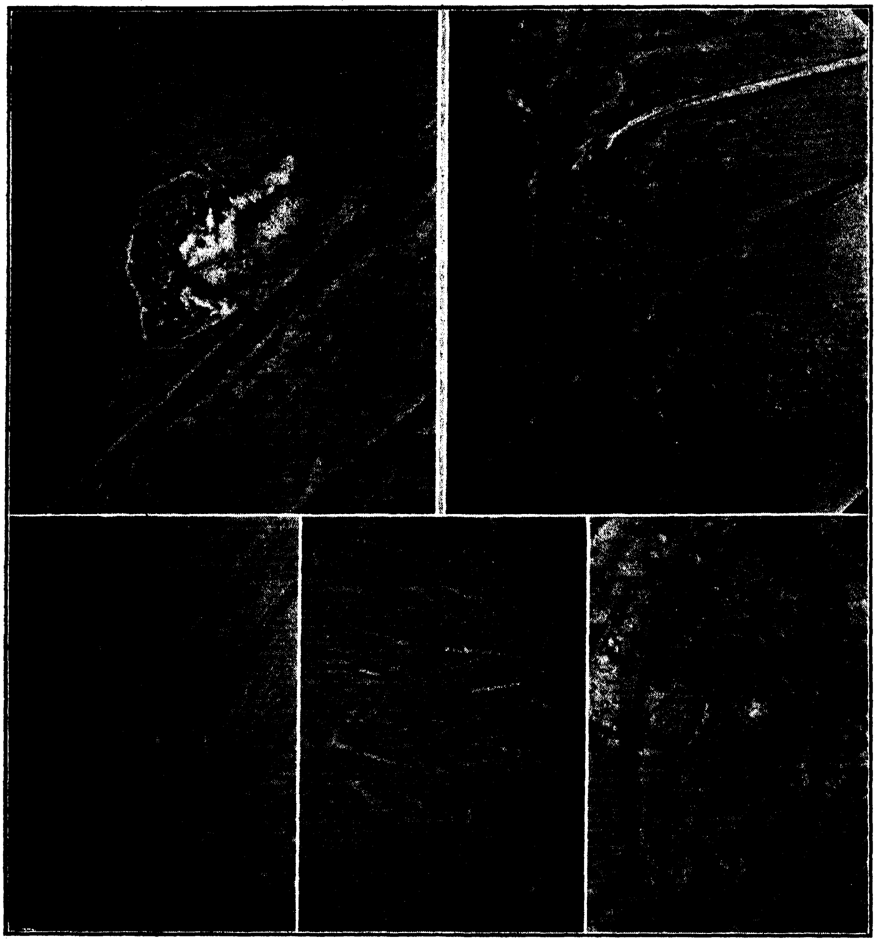


FIGURE 5. (A) Epidermal cell of mosaic-diseased petunia showing speckling and striations developing in crystals under influence of dilute HCl. (B) Epidermal cells of mosaic-diseased Turkish tobacco. Note various stages of reaction affected by different rates of penetration of the acid. Crystalline deposit in trichome unaffected, cell at base of trichome packed with needles. (C) Epidermal cell of mosaic-diseased Turkish tobacco showing cells filled with needle crystals precipitated by addition of dilute HCl. (D) Needle crystals prepared from virus extract according to Stanley's method. (E) Needle crystals in cell of glandular hair of tobacco after addition of HCl. ×640.

rapidly, water should be run under the cover slip immediately. With a little experimenting the reaction can be reasonably controlled. The pH of the acid used should not exceed 1.3 and if a single drop is added a good reaction is generally obtained within 5 to 10 minutes.

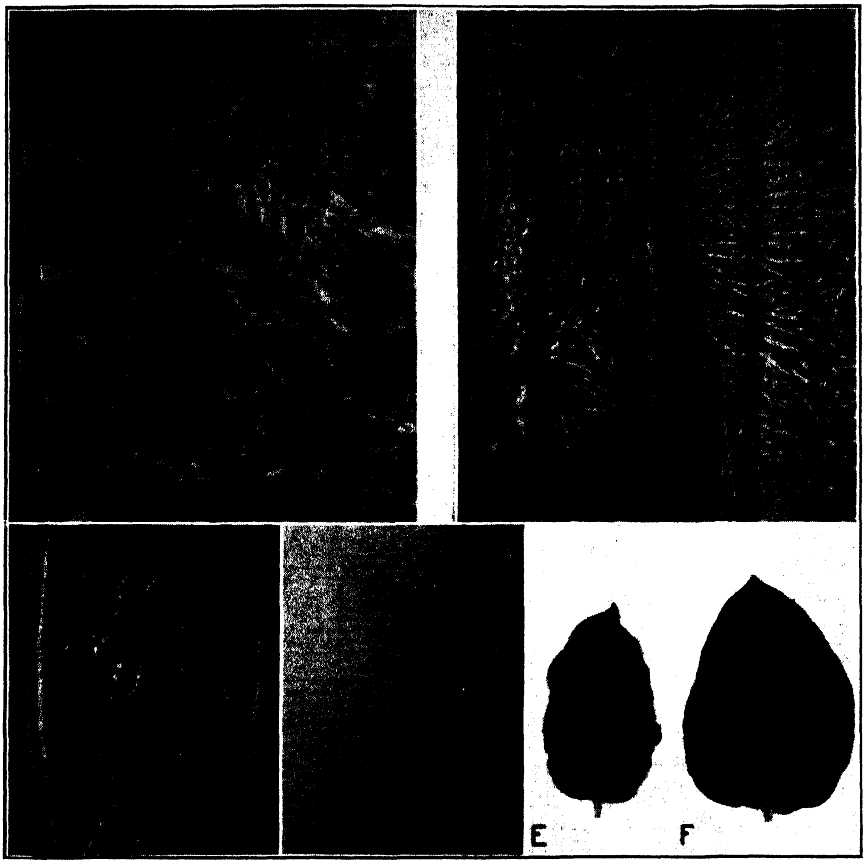


FIGURE 6. (A) Trichome of mosaic-diseased petunia after addition of dilute HCl. (B) Epidermal cells of mosaic-diseased Turkish tobacco with same acid added. Note needle crystals in cells where plates were present before addition of HCl. (C) Trichome from mosaic-diseased Turkish tobacco after addition of dilute HCl. Note nucleus, X-body at left of nucleus, and needle crystals filling the cell. ×950. (D) Epidermal cell of similar tissue to which excess of acid was added. Note X-body intact after dissolution of needle crystals. (E) Leaf of Turkish tobacco affected with a mottling strain of tobacco virus. (F) Leaf affected with Holmes' attenuated tobacco-virus strain in which the mottling symptoms are masked. Note small yellow spot in center at right of midrib. A, B, D ×630.

In Figure 6 C, a tobacco trichome cell to which acid had been added shows a rounded coagulated host-nucleus and an X-body with several refractive granules, at the left of the nucleus. The entire cell is filled with

needle crystals. Figure 6 D is an epidermal cell which originally was filled with needle crystals; but when an excess of acid was added to this cell, the crystalline material went into solution, leaving the X-body intact, indicating that the nature of the two substances is not identical.

The behavior of the crystalline material at both ends of the pH range was studied carefully. Solutions of increasing acidity were added to strips of diseased tissue and the effect was carefully observed under the microscope. The same procedure was adopted with solutions of increasing alkalinity. It was determined that solutions of acid of approximately pH 1.0 dissolved the crystalline material, while solutions of sodium hydroxide of 11.77 caused the material to dissolve without transformation to the needle form. Subsequent adjustment of the reaction to neutral failed to recrystallize the dissolved material. This point was considered significant in the identification of the crystalline material as a source of Stanley's needles, inasmuch as the crystalline protein, prepared from virus extracts, becomes denatured at approximately pH 1.0 and pH 11.0 to 11.8 (25, 29).

As control experiments, strips of tissue from Turkish tobacco plants affected separately with potato X virus and the ring-spot virus, and strips of healthy tissue from the same species of plant, were all treated in the same manner as the tobacco-mosaic infected tissue. The intracellular precipitation of needle crystals was not obtained in a single instance, although coagulation of the cytoplasm was observed, indicating the penetration of the acid.

Studies were made of three strains of tobacco-mosaic virus. The aucuba-strain of Bewley & Corbett (5), probably the same as Johnson's tobacco virus 6, the ordinary tobacco-mosaic virus, Johnson's tobacco virus 1 (19) and Holmes' attenuated strain (15). Tissue affected with the aucuba strain which produces the most intense chlorosis of the three strains, contained more crystals than tissue from ordinary tobacco-mosaic infections, while the attenuated strain which induces no mottling in the plant showed a general absence of crystalline material and X-bodies as well. Figure 6 E is a leaf removed from a plant affected with a mottling strain of tobacco mosaic. Figure 6 F is a leaf taken from a plant affected with a strain of tobacco virus so attenuated by Holmes that the mottling symptoms are masked. When acid was added to strips of epidermis taken from the midrib of the leaf, no crystallization of needles was obtained. In Figure 6 F, a small spot is visible in the middle of the leaf at the right of the midrib. Such yellow spots were observed first by McKinney (20) in ordinary tobacco-virus mosaic and were later described as characteristic of this attenuated strain by Holmes (15). Jensen isolated a number of yellow mosaics from such chlorotic areas (18). If the epidermis is removed in the region of this yellow spot, crystals typical of the other two strains are found in the cells. If acid is added to such a strip, the needles begin to form

in the crystalline material and very gradually spread to the neighboring cells in which no large crystalline plates or deposits were previously observed comparable to those found in chlorotic tissue.

DISCUSSION

From these results it seems justifiable to assume that the source of Stanley's crystallizable protein is the intracellular crystalline deposits just described in this article. While Dufrenoy & Dufrenoy (9) have figured crystalline inclusions in mosaic-diseased tobacco cells as "needle-like tyrosin or plate-like leucine" and Smith (24) has contributed to our knowledge of the solubility of the crystalline material, no detailed microchemical investigation of the crystals has been reported in the literature. The possibility that the crystalline material in the living cell is more complex than Stanley's needle crystals should not be overlooked. Also, the nature of the reaction involving the transformation of the plates to the needles upon the addition of acid or salts provides an interesting problem for future investigation.

Since the intracellular crystalline material of the living cell appears to be the source of Stanley's crystalline protein, it is of some interest to consider what factors are responsible for the presence of such large crystalline deposits in tissue affected with a virulent strain and for their relative absence in the case of the attenuated strain. The association of crystalline inclusions with chlorosis has long been recognized but the exact relation between the two has not been determined. If the virulence of a strain of tobacco virus is judged by the ability to induce chlorosis in a given host, then the three strains of virus in Turkish tobacco described in this article would be arranged as follows, in order of diminishing virulence (a) the aucuba strain, (b) the ordinary tobacco-virus strain, and (c) the attenuated one. Crystalline inclusions would then indicate virulence. A more restricted use of the term virulence denotes the power of invasiveness exhibited by an infectious agent. It has been shown by Holmes (16) that the strain of tobacco virus attenuated by him is characterized by a loss of the invasiveness exhibited by the original strain. According to either criterion, the attenuated strain would be considered the least virulent of the three. The most marked difference between the other two strains lies in the degree of intensity of the yellowing. Holmes has also demonstrated that the ability to induce yellowing of the chlorophyll and invasiveness are separate attributes. To be specific, yellow strains isolated from chlorotic spots in leaves affected with the attenuated strain, lack invasiveness in common with the strain whence they were derived. But yellow strains isolated from localized chlorotic spots on leaves affected with the ordinary tobacco-virus strain, possess that invasiveness. Both yellow strains, irrespective of their source, are capable of inducing a marked chlorosis. But

since the tissue in the region of yellow spots occurring on leaves infected with the attenuated strain contains deposits of crystalline material characteristic of the chlorotic areas in the mottled pattern produced by infections with the other two strains of virus, the presence of crystals is obviously independent of virulence as determined by the invasive power of the strain present in these limited chlorotic areas. Since the large yield of crystalline protein obtained by isolation from virus extract of the attenuated strain is out of proportion to the crystalline material present in the total yellow-spot area, it suggests that actual crystallization of the protein in the living cell may be dependent upon having a sufficiently high concentration of the crystallizable material.

Holmes (15) compared the virus concentration of a distorting, mottling strain of tobacco virus with that of an attenuated, masked one, after multiplication of the two strains separately in Turkish tobacco subjected to the same conditions. The results of his determinations indicate that the more virulent strain reached the higher virus concentration. Therefore, there is some evidence for assuming that in the attenuated strain the virus does not reach a sufficiently high concentration to produce a general crystallization of the protein in the living infected areas, but that the concentration of virus may be greater in the localized yellow spots, where crystals are present. It will be interesting to compare the virus concentration of plants affected with the attenuated strain with that of plants affected with the non-invasive yellow strains derived from the attenuated strain. In the case of bacterial infections, the more virulent strains usually proliferate more rapidly in the invaded host tissue. By analogy then, the yellow strain of the attenuated virus would be more virulent than the strain from which it was derived.

The fact that large deposits of intracellular crystalline material are characteristic of tobacco-mosaic virus and do not occur in other viruses affecting tobacco, may also be a question of concentration of the protein. Recent experiments by Stanley & Wyckoff (30) have shown that extracts of the cucumber, ring-spot, and potato X viruses, multiplied in the same host species, Turkish tobacco, give a low yield of high-molecular-weight protein when subjected to concentration in the ultracentrifuge. There is a correspondingly low concentration of virus, also, as determined by the dilution of virus extract capable of inducing disease in a susceptible host.

At this point it is interesting on the basis of the evidence at hand, to discuss the relationship between crystalline tobacco-virus protein, antigen, and virus. Are they one and the same protein or different substances? That a close correlation exists between antigen and virus has already been demonstrated (2, 6, 7). That there is a similar close correlation between the crystalline protein and virus has also been shown (28). There seems little doubt that a correlation also exists between crystalline protein and

antigen. Certainly, the close association of the tobacco-virus protein with virus is undeniable. Additional evidence has also been presented (4, 30) which suggests that characteristic high-molecular-weight proteins may be present in all virus extracts. One of the most important questions to be considered at the present moment seems to concern the source of this crystalline protein.

SUMMARY AND CONCLUSIONS

1. The procedure for isolating crystalline tobacco-virus protein from virus extract according to the method of Stanley is briefly outlined. Investigations corroborating Stanley are cited.

2. The isolation of Stanley's crystalline tobacco-virus protein is reported from *Nicotiana tabacum* L. var. Turkish, affected separately with ordinary tobacco virus and Holmes' attenuated strain, from air-dried Turkish tobacco, from *N. tabacum* L. var. White Burley, from *Solanum nigrum* L. var. *nodiflorum* affected with the aucuba strain, and from *Petunia* sp. affected with the ordinary tobacco-virus strain.

3. The tobacco-virus protein is reported isolated from streak-virus extract, a combination of potato X and tobacco virus. The use of the serum-precipitin reaction in detecting the possible presence of inactive potato X virus in a solution of tobacco-virus protein is demonstrated.

4. Experiments with antiserum to streak extract are described and from the results it is concluded that the specificity of the precipitin reaction for the two viruses is not altered by multiplication of potato X and tobacco virus simultaneously in the same plant.

5. Crystalline deposits, associated with chlorosis, and present in the living mosaic-diseased cell are described in detail and illustrated by microphotographs. X-bodies are briefly considered.

6. Transformation of the crystalline plates to needle crystals by the addition of dilute acid or salt to the water mount of living diseased tissue is fully described and illustrated.

7. The transformation of the crystalline plates to needles is reported in all hosts tested, namely, *Nicotiana tabacum* L. varieties Turkish and White Burley, *Petunia* sp., *Lycopersicon esculentum* Mill., *Capsicum* sp., and *Solanum nigrum* L. var. *nodiflorum*. Dilute sulphuric, hydrochloric, acetic, nitric and saturated magnesium sulphate solutions are the reagents listed in effecting the transformation.

8. It is suggested that the intracellular crystalline plates may be more complex in chemical constitution than Stanley's crystalline tobacco-virus protein.

9. It is concluded that the intracellular crystalline deposits are the source of Stanley's crystalline tobacco-virus protein, because: A. The two crystalline compounds are present in large amounts. B. There is a striking

similarity in the gross appearance of the needles precipitated in the cell and isolated from virus extract. C. The acidity and alkalinity at which Stanley reports denaturation of the protein corresponds closely to the reactions at either end of the pH range at which the intracellular crystals go into solution and are not subsequently recrystallizable.

10. It is concluded that concentration is an important factor in the intracellular crystallization of tobacco-virus protein.

LITERATURE CITED

1. BAWDEN, F. C., N. W. PIRIE, J. D. BERNAL, and I. FANKUCHEN. Liquid crystalline substances from virus-infected plants. Nature [London] **138**: 1051–1052. 1936.
2. BEALE, HELEN PURDY. The serum reactions as an aid in the study of filterable viruses of plants. Contrib. Boyce Thompson Inst. **6**: 407–435. 1934.
3. ——— Possible relationship of Stanley's crystalline tobacco-mosaic-virus material to intracellular inclusions present in virus infected cells. Contrib. Boyce Thompson Inst. **8**: 333. 1936. (*Also in* Phytopath. **27**: 122. 1937.)
4. BEARD, J. W., and RALPH W. G. WYCKOFF. The isolation of a homogeneous heavy protein from virus-induced rabbit papillomas. Science **85**: 201–202. 1937.
5. BEWLEY, W. F., and W. CORBETT. "Mosaic" disease of the tomato. Nursery & Market Gard. Indus. Develop. Soc. Ltd. Exp. & Res. Sta. Ann. Rept. **1927**: 51–59. 1928.
6. CHESTER, KENNETH S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. **24**: 1180–1202. 1934.
7. ——— The antigenicity of the plant viruses. Phytopath. **25**: 702–714. 1935.
8. CLINCH, PHYLLIS. Cytological studies of potato plants affected with certain virus diseases. Sci. Proc. Roy. Dublin Soc. **20**: 143–172. 1932.
9. DUFRENOY, J., and M. T. DUFRENOY. Cytology of plant tissues affected by viroses. Phytopath. **24**: 599–619. 1934.
10. GOLDSTEIN, BESSIE. Cytological study of living cells of tobacco plants affected with mosaic disease. Bull. Torrey Bot. Club **51**: 261–274. 1924.
11. ——— A cytological study of the leaves and growing points of healthy and mosaic diseased tobacco plants. Bull. Torrey Bot. Club **53**: 499–600. 1926.
12. GRANT, THEODORE J. The host range and behavior of the ordinary tobacco-mosaic virus. Phytopath. **24**: 311–336. 1934.
13. HOGGAN, ISMÉ A. Cytological studies on virus diseases of solanaceous plants. Jour. Agric. Res. **35**: 651–671. 1927.
14. ——— Further studies on aphid transmission of plant viruses. Phytopath. **21**: 199–212. 1931.
15. HOLMES, FRANCIS O. A masked strain of tobacco-mosaic virus. Phytopath. **24**: 845–873. 1934.
16. ——— Comparison of derivatives from distinctive strains of tobacco-mosaic virus. Phytopath. **26**: 896–904. 1936.
17. IWANOWSKI, D. Über die Mosaikkrankheit der Tabakspflanze. Zeitschr. Pflanzenkrankh. **13**: 1–41. 1903.
18. JENSEN, JAMES H. Isolation of yellow-mosaic viruses from plants infected with tobacco mosaic. Phytopath. **23**: 964–974. 1933.
19. JOHNSON, JAMES. The classification of plant viruses. Wisconsin Agric. Exp. Sta. Res. Bull. 76. 16 pp. 1927.
20. MCKINNEY, H. H. Virus mixtures that may not be detected in young tobacco plants. Phytopath. **16**: 893. 1926.

21. MATSUMOTO, TAKASHI, and KÔETSU SOMAZAWA. Immunological studies of mosaic diseases. 1. Effect of formolization, trypsinization and heat-inactivation on the antigenic properties of tobacco mosaic juice. Part I. Jour. Soc. Trop. Agric. **2**: 223–233. 1930. (*Also in* Phytopath. Lab. Taihoku Imp. Univ. Contrib. 7. 1930.)

22. PURDY, HELEN A. Immunologic reactions with tobacco mosaic virus. Jour. Exp. Med. **49**: 919–935. 1929.

23. RAWLINS, T. E., and JAMES JOHNSON. Cytological studies of the mosaic disease of tobacco. Amer. Jour. Bot. **12**: 19–32. 1925.

24. SMITH, FANNY FERN. Some cytological and physiological studies of mosaic diseases and leaf variegations. Ann. Missouri Bot. Gard. **13**: 425–484. 1926.

25. STANLEY, W. M. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science **81**: 644–645. 1935.

26. ——— Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. Phytopath. **26**: 305–320. 1936.

27. ——— The inactivation of crystalline tobacco-mosaic virus protein. Science **83**: 626–627. 1936.

28. ——— Chemical studies on the virus of tobacco mosaic. IX. Correlation of virus activity and protein on centrifugation of protein from solution under various conditions. Jour. Biol. Chem. **117**: 755–770. 1937.

29. ——— Crystalline tobacco-mosaic virus protein. Amer. Jour. Bot. **24**: 59–68. 1937.

30. STANLEY, W. M., and RALPH W. G. WYCKOFF. The isolation of tobacco ring spot and other virus proteins by ultracentrifugation. Science **85**: 181–183. 1937.

31. TAKAHASHI, WILLIAM N., and T. E. RAWLINS. Stream double refraction of preparations of crystalline tobacco-mosaic protein. Science **85**: 103–104. 1937.

PLANT GROWTH UNDER CONTINUOUS ILLUMINATION FROM SODIUM VAPOR LAMPS SUPPLEMENTED BY MERCURY ARC LAMPS

John M. Arthur and Edward K. Harvill

In a former publication (5) the growth of buckwheat plants under Mazda, neon, sodium, and mercury vapor lamps was compared for short periods of 8 to 11 days. The sodium vapor lamp was found to be the most efficient in the dry weight production of plant tissue of all the lamps tested when equal energy values in the visible region were compared. Considering the high efficiency of light output in relation to current consumption (45 lumens per watt) this lamp is outstanding as a promising light source now available for growing plants. Further tests reported herewith indicate that the lamp is not an ideal light for growing plants continuously over long periods of time unless supplemented by short exposures each day to other more complete light sources. However, when other sources are used for a brief period each day along with sodium vapor applied continuously, the light seems to be as good in quality as sunlight for geranium (*Pelargonium zonale* Willd.), cotton (*Gossypium hirsutum* L.), begonia (*Begonia tuberosa* Hort.), buckwheat (*Fagopyrum esculentum* Moench.), and other species of plants. The leaves of plants grown under this combination of lamps have in general a very fine dark green color and contain more chlorophyll than those grown in sunlight. Geranium, a plant which does not withstand continuous exposure to Mazda lamps, grows well and flowers well under the sodium and mercury lamp combination, while tomato, when exposed continuously, does not grow well under either type of lamp.

LIGHT SOURCES

A new arrangment of four 10,000-lumen sodium vapor lamps was designed for plant work.[1] In this arrangement the lamps are mounted one on each side of a square iron frame. A photograph of this lighting unit is shown in Figure 1. The frame is approximately 24 inches square over all dimensions. Each lamp is fitted with a separate aluminum reflector, the outside edge of which projects down about three and one-half inches below the glass vacuum jacket which covers the lamp. This arrangement concentrates the entire light output of the four lamps in a square of approximately four feet. Each lighting unit of four sodium vapor lamps uses approximately 1000 watts of current and will light 16 square feet of area effectively. The tips of the plants are normally kept about 12 to 15 inches

[1] These lamps were furnished by the General Electric Vapor Lamp Co., Hoboken, New Jersey.

FIGURE 1. An arrangement of four 10,000-lumen sodium vapor lamps designed especially for plant work.

below the lamps, although on account of the low heat output, plants can be grown much closer than this without injury. A small 85-watt capillary mercury arc lamp, type H-3, was mounted in the center of the square formed by the sodium lamps. It was fitted with an aluminum reflector of the same shape and style as the R.L.M. "Ivanhoe" type. In earlier tests the 400-watt, type H-1, mercury arc was used, but this lamp has much more energy than is desirable, and is inferior in quality of light for plant work. The characteristics of the mercury lamps have been discussed by Buttolph (6). The small lamp, type H-3, has an operating temperature of over 5000° K. (7), and differs from most mercury arcs in that it has a continuous spectrum similar to sunlight over which is superposed a relatively strong bright line spectrum of mercury. It, therefore, has considerable energy in the red region of the spectrum, as well as a high output in the blue-violet and is essentially a complete light source. The lamp has a life of 500 hours, but since it is used for only two hours a day in this work, it should last for approximately eight months. Two of the sodium vapor lamps burned continuously in these tests from April 27 to December 2, a total of more than 5000 hours. The four sodium vapor lamps consume one kilowatt per hour, so that with current furnished at two cents per K.W.H., the total cost per 24-hour day for current (burning continuously) is approximately 50 cents.

METHODS AND RESULTS

The plants were grown in a basement room. No attempt was made to control temperature or humidity during these tests. The temperature held near 70° F., but on cold nights dropped a few degrees below this value. Preliminary tests started in April showed that plants grew well for a time under the sodium vapor lamps, but that after a month or two months' continuous exposure, many leaves became yellow and dropped off. This was especially true of geranium and cotton plants. A more detailed discussion of these plants is of interest.

On August 1, eight seedling geranium plants which had become pot-bound were removed from the greenhouse and repotted in larger pots using a rich soil. Four of these were returned to the greenhouse and four were placed under the sodium lamps and exposed continuously to sodium light only. By August 20 many new leaves had developed, but all of these produced under the sodium vapor light were albino—wholly free from green pigment. A photograph of one plant along with the control which was kept in an ordinary greenhouse is shown in Figure 2 A. Chlorophyll developed on the younger leaves in a day or two, following short exposures of the plants to diffuse daylight and to Mazda lamps, while the plants were being photographed. Chlorophyll never developed, however, on the older leaves even when kept continuously in the greenhouse. Attempts at

repeating this experiment gave plants with some albino and some green leaves, but leaf production was very slow on these plants, due probably to the fact that they had not been held back as much as the first by small pots. During repeated tests and in every case if the continuous exposure to the sodium lamps was sufficient, geranium plants could be "run down" to only one or two yellow leaves. This usually required about two months. At this time if exposed to the capillary mercury lamp for only two hours per day along with continuous exposure to sodium light, the plants recovered rapidly. New dark green leaves developed and the plant came into flower again in a short time. This is illustrated in Figure 2 B, a plant which was grown under sodium vapor lamps only from October until January 9. It was then exposed two hours each day to the capillary mercury lamp and the photograph was taken on January 27 when four new leaves had developed. This plant had degenerated to the single albino leaf shown in the photograph before the first exposure to the mercury lamp. Figure 2 C shows the same plant on February 23 when many more leaves had grown and a flower was opening. The plant in Figure 2 D was exposed to sodium vapor light continuously supplemented with two hours each day from the capillary mercury light from January 31 until February 23, while the plant in Figure 2 E was exposed similarly except that it was kept in a dark room for two hours each day, while the other plant was exposed to the mercury lamp. It received, therefore, only sodium vapor and no mercury vapor light. Many of the leaves had already dropped from the plant shown in Figure 2 E, while others were yellowing and were without green pigment. This degeneration process is very slow and it will require several weeks' further exposure to reach complete defoliation. The plant in Figure 2 D developed more leaves, increased greatly in depth of green color, and came into flower. It is interesting that this brief exposure to the mercury lamp each day produces such fine growth, development and flowering, whereas without it, the plant gradually degenerates to only a leafless stem.

Cotton plants respond in a similar way. The plant in Figure 3 A was grown from a small seedling placed under sodium vapor lamps on April 27. This light was supplemented from time to time with a high pressure mercury lamp (type H-1) until October 15, when it was exposed to sodium vapor only until January 9. On this last date it had degenerated to seven yellow mottled leaves. A single leaf photographed at this time is shown as Figure 3 B. Photograph 3 A was taken on January 15 after this plant had received an exposure of two hours each day for seven days to the capillary mercury lamp (type H-3) in addition to continuous exposure to the sodium vapor lamp. By this date the plant had already grown a number of new dark green leaves. A few days later the plant was repotted and it continued to grow very rapidly with the additional two hours of light

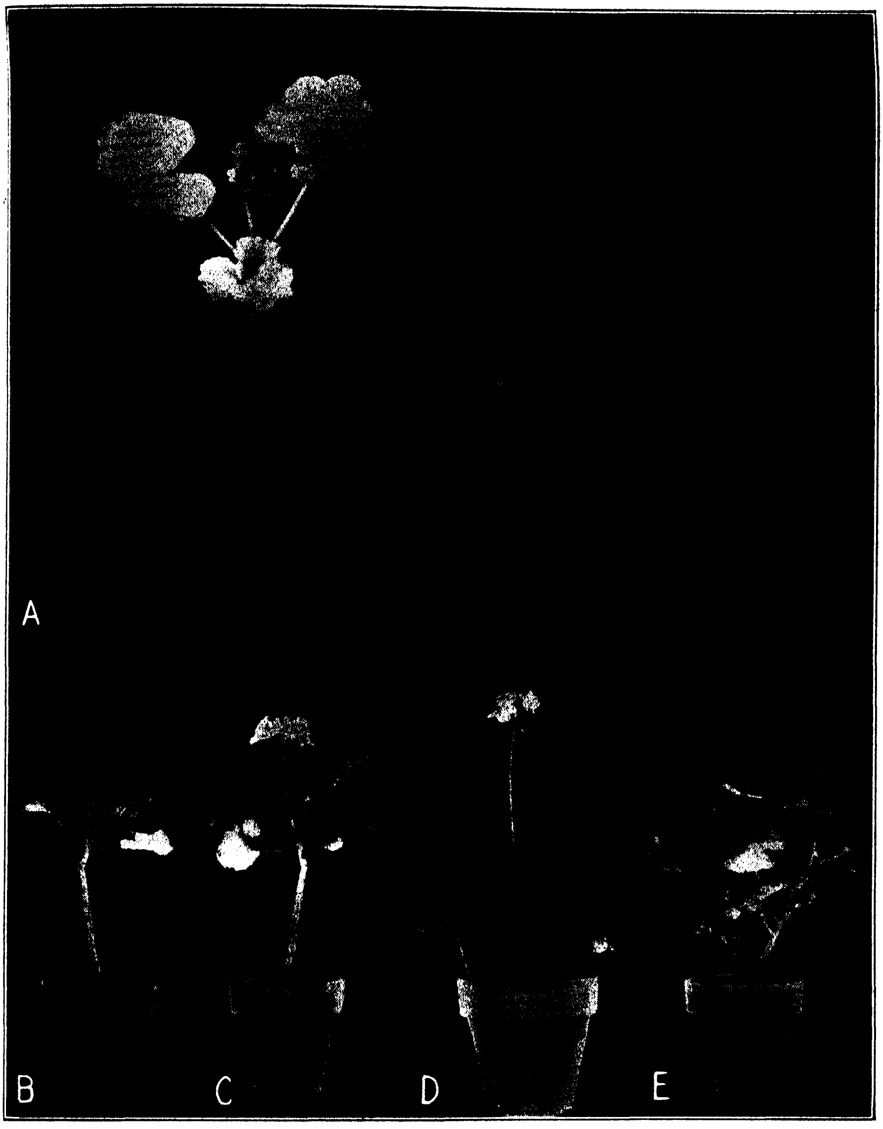


FIGURE 2. Geraniums. A. Left, albino leaves produced by rapid growth under sodium vapor light alone; right, control plant from greenhouse. B. Plant which has been run down by continuous sodium vapor regenerating again when exposed two hours per day to mercury lamp. C. Same plant as B. about a month later. D. Plant which has been exposed to both sodium vapor and mercury vapor. E. Plant exposed similarly to D except received no mercury vapor light.

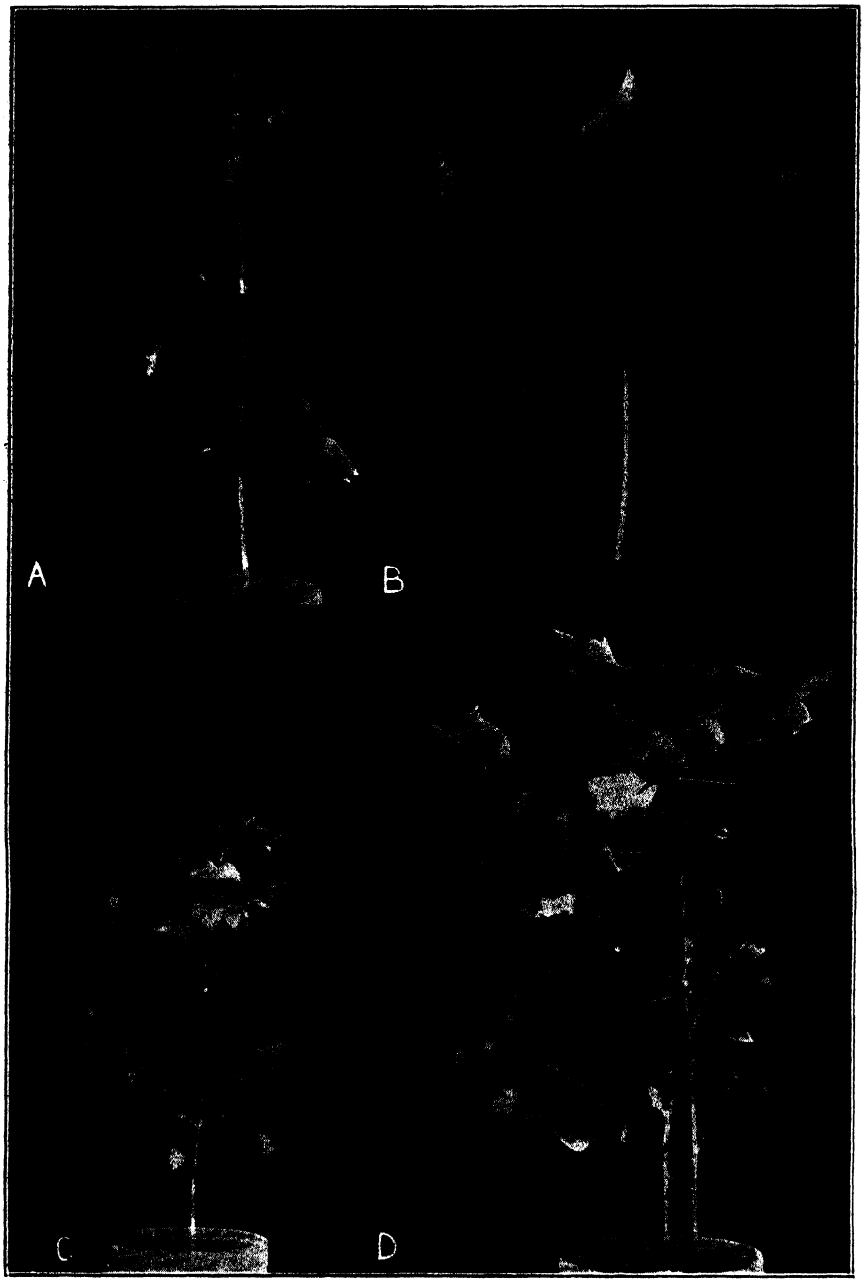


FIGURE 3. Cotton. A. Plant run down by continuous sodium vapor light partially regenerated by exposure each day to mercury lamp. B. Single leaf showing typical injury. C. Same as A, 12 days later. D. Same as C, 27 days later.

each day from the capillary lamp. It was photographed again on January 27 (Fig 3 C). The plant, almost completely regenerated in this brief period of time, had developed several flowers and had set one boll. It was photographed again on February 23 (Fig. 3 D), after it had produced 10 flowers and had set several bolls. The cotton plant like the geranium would have failed completely if it had been allowed to continue with the sodium vapor lamps alone, but with the short exposure to other wave bands of light each day, something was supplied, or some process proceeded which permitted vigorous growth and development. This response of plants seems all out of proportion to the energy involved—that is, a two-hour exposure to an 85-watt lamp. No doubt a much shorter exposure would be sufficient, but how small an exposure and what regions of the spectrum are effective must be determined in further study. It is not surprising that a light source, such as the sodium vapor lamp, with practically all of its visible energy in a single line concentrated around 588 mμ should fail in certain very definite respects as an entirely suitable source for growing plants. The odd thing is that only a short exposure of an essentially complete light source such as the capillary lamp is sufficient to produce, when applied along with the sodium light, such excellent development of many plants.

The sodium-mercury light source is not ideal, however, for all plants. The tomato plant grows very tall and spindling under it as is shown in Figure 4 A. In a previous publication (2) it was shown that both geranium and tomato failed to withstand continuous light from the Mazda lamp. The combination light source described herewith seems excellent for geranium but very poor for tomato. Tomatoes are known to grow well within the Arctic Circle under continuous sunlight, so that no doubt this plant will eventually respond well to continuous illumination when its essential wave bands of light are found.

Leaf color on most plants grown under this lamp combination appears excellent. Table I shows the percentage composition of chlorophyll A and B on a green weight basis of buckwheat leaves grown under both sodium vapor light alone and in combination with two hours of mercury light each day in comparison with similar leaves grown in sunlight. The method of Guthrie (8) was followed in the analyses. The data show an increase in total chlorophyll in both cases where artificial light was used, with slightly greater chlorophyll in greenhouse leaves in July than in February. The highest ratio of A to B was shown in leaves taken from the greenhouse in July and the lowest in leaves taken from the greenhouse in February. Since the buckwheat plants are grown only a few weeks in each condition, they do not show marked differences in leaf color production when the mercury lamp is used. There is a correspondingly small difference in the amount of chlorophyll. Other plants which grow much more slowly, such as geranium and cotton, and develop yellow mottled leaves without

the mercury lamp would no doubt show a much greater difference in chlorophyll.

Tuberous rooted begonias, gardenias, and buckwheat all grow well and flower well under the sodium vapor lamps when supplemented with the capillary lamps. Snapdragon flowers unusually well but the stems

FIGURE 4. Various plants grown under continuous sodium vapor light supplemented by mercury vapor two hours per day. A. Tomato. B. Snapdragon. Left, artificial light; right, greenhouse control. C. Gardenia. D. Tuberous rooted begonia, left, greenhouse; right, artificial light. E. Hyacinths.

produced are weak, brought about by a too rapid elongation in comparison with the increase in diameter of the stem. A photograph of three of these plants is included as Figure 4 B, C, and D. The sodium-mercury combination lamps are especially effective in flower production on gardenias and snapdragon. Gardenia plants, placed under the lamps, which had been

grown in a greenhouse supplemented by Mazda lamps (4) each night greatly increased the rate of opening of buds. The young gardenia plant shown as Figure 4 C was transferred from a cool greenhouse which received two to six hours of light from 500-watt Mazda lamps each night to the light room on March 15, where it received continuous sodium vapor light plus two hours of mercury light each day. The photograph was taken on March 26 when the sixth flower had opened during this continuous exposure of eleven days. This plant had previously produced only one flower in the greenhouse, but had developed 11 or 12 large buds. It was also found possible to take a gardenia plant from a hot greenhouse (above 70° F.)

TABLE I

CHLOROPHYLL ANALYSES OF BUCKWHEAT LEAVES GROWN UNDER CONTINUOUS ARTIFICIAL LIGHT AS COMPARED WITH SIMILAR LEAVES GROWN WITH SUNLIGHT. PERCENTAGE GREEN WEIGHT

	Chlorophyll A	Chlorophyll B	Total	Ratio $\frac{A}{B}$
Na vapor light alone	0.220	0.048	0.268	4.54
Sampled July 22, 1936	0.212	0.047	0.259	4.47
Greenhouse—sunlight	0.141	0.030	0.170	4.76
Sampled July 22, 1936	0.142	0.030	0.172	4.68
Na vapor light & 2 hrs.				
Hg vapor each day	0.212	0.049	0.261	4.32
January 28, 1937	0.211	0.046	0.256	4.59
Greenhouse—sunlight	0.101	0.025	0.126	4.01
February 3, 1937	0.086	0.021	0.108	4.03

in which flower buds remain dormant and develop buds rapidly by keeping it in a cold room (50° F.) each night and exposing it to the lamps each day until the buds were well developed (3). The plant was then held continuously under the sodium plus mercury lamp combination until the flowers opened. This plant treatment was started on January 15 and the first flower opened on March 26 from buds which had been developed entirely under artificial light. The leaf color under this treatment was excellent whereas with sodium vapor alone, leaf color is poor. That is, the leaves develop a yellow-green mottle which is characteristic of many plants exposed to the sodium vapor lamp alone. It is now believed entirely possible to take gardenia plants at any season out of a warm greenhouse where the flower buds remain completely dormant and grow buds to the flowering stage in a period of approximately two and one-half months with artificial light only.

In Figure 4 E is shown a pot of hyacinth plants which were brought into flower without the aid of sunlight. These bulbs were started in a cool dark

room and grown until the plants were approximately two inches above the soil. They were then given artificial light during the day and returned to the cold room (50° F.) each night until the peduncles had grown sufficiently following which they were exposed continuously to the combination sodium-mercury lamps. The flowers were well formed but had a slightly paler shade of blue than those grown with sunlight. This is in contrast to the color of geranium flowers which seemed to produce a brighter red under the lamps than those grown with sunlight. Earlier work (1) has shown that the development of certain red anthocyanin pigments in plants is greatly favored by blue-violet and ultra-violet regions. The capillary mercury lamp has a high output in these regions and this may account for the increase in red pigments.

It should be stated, however, that this method can not at present compete commercially with sunlight since both lamps and current are costly while sunlight is generally available at little or no cost. It is of considerable interest to those who wish to grow a few plants without any sunlight at perhaps a minimum cost for current. This lighting combination has very little heat, so that it should prove useful to plant pathologists and others who wish to grow plants in glass enclosed cases where humidity and temperature can be controlled and in which plants can be grown fairly well and the effects of certain pathogenes can be studied. It is not known how long plants will continue to survive under such artificial light, or what plants, if any, will not survive. The cotton plant as outlined above has grown from a small seedling with only two leaves for 11 months under continuous artificial light only, and still appears to be in good condition. It is believed that this is a record for the growth of plants under continuous artificial light. In the future it may be found that other wave bands of light need to be added or subtracted for the production of some plants, such as the tomato, but after more than 13 years of experience with many types of arc, filament, and gaseous discharge lamps, the combination of sodium and capillary mercury vapor lamps is believed to be the most promising light source for growing plants when exposed continuously and in the complete absence of sunlight.

SUMMARY

1. Plants which grow well for a short time when exposed continuously to sodium vapor lamps were found to degenerate slowly, so that after a period of two months' continuous exposure only a few yellow leaves at the tip remain.

2. Such plants could be completely rejuvenated by two hours' exposure each day to the 85-watt capillary mercury vapor lamp when applied along with continuous sodium vapor lamps.

3. Excellent leaf color and flowering could be produced when plants

such as begonia, gardenia, cotton, geranium, buckwheat, and snapdragon were exposed continuously to sodium vapor lamps supplemented each day with two hours' exposure to the capillary mercury lamp. This light source was not satisfactory for tomato plants.

4. A new set of sodium vapor and capillary mercury lamps, when operated as outlined above, should burn continuously for approximately seven months. On account of the high efficiency of these light sources the cost for current at two cents per kilowatt is approximately fifty cents per 24-hour day, and one lighting unit will cover effectively at least 16 square feet, making an average cost of approximately three cents per square foot per day.

LITERATURE CITED

1. ARTHUR, JOHN M. Red pigment production in apples by means of artificial light sources. Contrib. Boyce Thompson Inst. **4**: 1–18. 1932.
2. ARTHUR, JOHN M., JOHN D. GUTHRIE, and JOHN M. NEWELL. Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**: 416–482. 1930. (*Also in* Contrib. Boyce Thompson Inst. **2**: 445–511. 1930.)
3. ARTHUR, JOHN M., and EDWARD K. HARVILL. Forcing flower buds in gardenia with low temperature and light. Contrib. Boyce Thompson Inst. **8**: 405–412. 1937.
4. ARTHUR, JOHN M., and L. C. PORTER. A new type of insulated greenhouse heated and lighted by Mazda lamps. Contrib. Boyce Thompson Inst. **7**: 131–146. 1935.
5. ARTHUR, JOHN M., and W. D. STEWART. Relative growth and dry weight production of plant tissue under Mazda, neon, sodium, and mercury vapor lamps. Contrib. Boyce Thompson Inst. **7**: 119–130. 1935.
6. BUTTOLPH, L. J. Mercury arcs of increased brightness and efficiency. Jour. Soc. Motion Pict. Eng. **28**: 43–49. 1937.
7. DUSHMAN, SAUL. The search for high efficiency light sources. Jour. Optical Soc. Amer. **27**: 1–24. 1937.
8. GUTHRIE, JOHN D. Effect of environmental conditions on the chloroplast pigments. Amer. Jour. Bot. **16**: 716–746. 1929. (*Also in* Contrib. Boyce Thompson Inst. **2**: 220–250. 1929.)

A STUDY OF ASCORBIC ACID AS AN INACTIVATING AGENT OF TOBACCO MOSAIC VIRUS[1]

MARY LOJKIN

INTRODUCTION

The effect of ascorbic acid on the infectious agents of several diseases of human beings and animals has been studied within the last few years. Jungeblut and Zwemer (19) produced complete inactivation of diphtheria toxin in vitro by means of ascorbic acid, and also succeeded in many cases in protecting guinea pigs against the fatal outcome of diphtheria intoxication by injections of suitable amounts of this vitamin. Results of a similar nature were reported by Polónyi (28), Gagyi (7), Harde (12), Greenwald and Harde (10), and Kligler (21). In a study of the effect of ascorbic acid on the development of various bacteria in synthetic nutrient media, Grooten and Bezssonoff (11) found that this vitamin inhibited the development of the whooping cough bacteria (*Bacillus pertussis*) but failed to produce any effect under similar conditions on the growth of all the other bacteria tested (*Micrococcus melitensis*, *Staphylococcus*, *Pneumococcus*, *Bacillus coli*, *B. paratyphosus*, *B. diphtheriae*, *B. tetani*, *B. sporogenes*, *Vibrio septicus*). Lominski (23) observed that ascorbic acid inactivated bacteriophages of *Staphylococcus*, *Bacillus coli*, *B. subtilis*, and *B. dysenteriae*. Jungeblut (17) was the first to study the effect of ascorbic acid on animal viruses. He found that multiple paralytic doses of poliomyelitis virus, when mixed with small amounts of ascorbic acid in vitro, were rendered non-infectious, as determined by intracerebral injections of such mixtures into rhesus monkeys. In a recent paper (18) this investigator also stated that a decrease in the injurious effect of this virus could be produced in some cases by daily injections of ascorbic acid into monkeys which had been previously inoculated with poliomyelitis virus.

In spite of the great importance of these properties of ascorbic acid, very little has been reported on the nature of the chemical reaction resulting in the inactivation of infectious agents by ascorbic acid. Lominski (23), and Grooten and Bezssonoff (11) suggested that the inactivating power of ascorbic acid is associated with its ability to act as a reducing agent. These workers did not investigate the nature of the reaction which occurred in the course of the inactivation of the infectious agents.

From the work of Tillmans, Hirsch, and Dick (40) and of Johnson (16) it is known that ascorbic acid can be oxidized to dehydroascorbic acid by mild oxidizing agents such as iodine, 2,6-dichlorophenolindophenol and

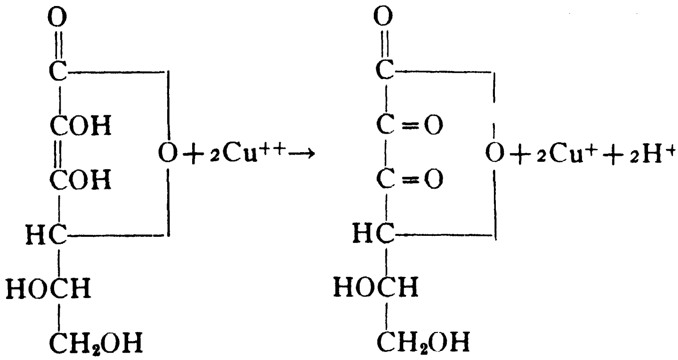
[1] This article was preprinted July 23, 1937.

hydrogen peroxide. This oxidation, which consists of the loss of two hydrogen atoms by each molecule of ascorbic acid, is reversible, the dehydroascorbic acid being easily reduced to ascorbic acid by means of hydrogen sulphide. Barron et al. (2) found that in acid solutions ascorbic acid is oxidized by molecular oxygen to its reversibly oxidized form, while in alkaline solutions the oxidation proceeds further to the irreversible stage, with the breaking down of the ascorbic acid molecule and formation of oxalic and l-threonic acids. Kellie and Zilva (20) suggested that the oxidation of ascorbic acid in neutral solutions proceeds first to the reversible state, and is followed subsequently by the irreversible oxidation. Mawson (25) and Kellie and Zilva (20) reported that oxidation of ascorbic acid in neutral solutions by atmospheric oxygen does not proceed easily in the absence of a catalyst. They found that small concentrations of copper ions have a strong catalytic effect on the oxidation of ascorbic acid, while some substances such as extracts of animal tissue (20), cysteine, cystine and glutathione (25) inhibit the autoxidation of ascorbic acid. This inhibiting effect, according to these investigators, is due to the ability of these agents to remove ionic copper from solution and prevent copper ions from catalyzing the oxidation of ascorbic acid. These results were confirmed by the work of Barron and coworkers (2, 3). Barron, DeMeio, and Klemperer (2) reported that in the absence of catalysts ascorbic acid is oxidized by oxygen only in alkaline solutions, the rate of oxidation increasing as the alkalinity increases from pH 8 to pH 10. According to these investigators, cupric ions appear to be rather unique in the capacity to catalyze the autoxidation of ascorbic acid; none of the other ions (manganese, nickel, cobalt, ferric, ferrous, calcium) which they tested possessed this property. These investigators advanced the hypothesis that the autoxidation of ascorbic acid under the catalytic effect of cupric ions proceeds with the formation of hydrogen peroxide as an intermediate product. They suggested that the cupric ions are reduced by ascorbic acid to the cuprous state; the cuprous ions are then oxidized to cupric ions with simultaneous formation of hydrogen peroxide, which is subsequently split into oxygen and water, as represented by the equations on the next page.

The autoxidation of ascorbic acid is also catalyzed by an enzyme "hexoxidase," which Szent-Györgyi (39) obtained from cabbage leaves (*Brassica oleracea* L.). Under the catalytic effect of this enzyme ascorbic acid undergoes reversible autoxidation.

In a preliminary paper (22) the author reported that under certain conditions ascorbic acid produces complete inactivation of tobacco mosaic virus *in vitro*. Crystalline proteins possessing the properties of tobacco mosaic virus have been obtained by Vinson (41, 42) and by Stanley (33, 34, 35) from Turkish tobacco plants infected with this virus. Stanley (34)

(1)



(2) $2Cu^{+} + 2H^{+} + O_2 \rightarrow 2Cu^{++} + H_2O_2$

(3) $2H_2O_2 \rightarrow 2H_2O + O_2$

and Loring and Stanley (24) reported their crystalline products to have a nitrogen content of approximately 16 per cent, an optical rotation of $[\alpha]^{20}_{D} = -0.43$, an isoelectric point of 3.3 as determined by electrophoresis, and a molecular weight of the order of 17,000,000 as determined by Svedberg's ultra-centrifugal method. Suspensions of this crystalline protein applied to the outside surface of the leaves of *Nicotiana glutinosa* L. plants induce characteristic tobacco mosaic lesions. Holmes (14) found that the relative infectivity of different virus suspensions can be estimated under carefully controlled conditions by the number of lesions which appear on the leaves of that host.

The present investigation was undertaken with the purpose of studying the nature of the chemical reaction associated with the inactivation of tobacco mosaic virus by ascorbic acid. Preliminary work (22) indicated that this inactivation was closely related to the oxidation of ascorbic acid by atmospheric oxygen. Both the autoxidation reaction and the inactivation reaction were catalyzed by cupric ions. In further studies the course of the oxidation of ascorbic acid accompanying the inactivation of the virus under different experimental conditions was followed by simultaneous measurements of the relative infectivity of the virus and of the amount of ascorbic acid oxidized in the virus-ascorbic acid systems.

EXPERIMENTAL

The inactivation reaction in virus-ascorbic acid systems was studied under different experimental conditions. The effects of various concentrations of ascorbic acid, the hydrogen ion activity of the reacting systems, the saturation of the virus-ascorbic acid systems with different gases, and the presence of certain substances which might possess catalytic or in-

hibiting effects on the reaction were investigated. In all of these experiments, unless otherwise stated, cupric ions, which acted as a catalyst for the autoxidation of ascorbic acid, were present at a concentration of 10^{-4} mg. per cc., and the reaction mixtures were brought to pH 6 by the use of a phthalate buffer. The temperature of the reacting systems was maintained at 25° C.

Crystalline virus material prepared by Stanley's method (33, 34, 35) was used in most of the experiments. A few experiments were performed with Vinson's (41, 42) crystalline virus material and with crude juice extracted from diseased plants. In all experiments the different virus preparations were used at concentrations matching in infectivity. The control or reference suspension of the virus contained 0.01 per cent of protein.

The relative infectivities of the virus in the virus-ascorbic acid systems were determined by inoculating *Nicotiana glutinosa* L. plants with the reaction mixtures. In view of the variations in the degrees of susceptibility to infection of different plants, and different leaves of the same plant, the infectivities of different samples were compared by the half-leaf method [Samuel & Bald's (31) modification of Holmes' method]. The reaction mixtures of the virus-ascorbic acid systems and the untreated control virus suspension were inoculated on the opposite halves of the same leaves of a number of plants and the average numbers of lesions produced per half leaf by the two different solutions were compared. When the differences obtained were not significant the conclusion was made that no inactivation occurred. A marked decrease in the average number of lesions produced by the treated virus as compared with the control was considered an indication of partial inactivation, while a total absence of lesions on the halves inoculated with the reaction mixtures indicated complete inactivation. In case the results of the half-leaf method indicated complete inactivation of a reaction mixture, this was further tested on a number of whole plants. Inoculation of the whole plants in determining complete inactivation has the advantage over the half-leaf method in minimizing the danger of contaminations.

In order to follow the changes which proceed in a solution containing the virus and the ascorbic acid, aliquots of the reaction mixture were pipetted out at short intervals during the progress of the reaction. Each of these aliquots was immediately titrated against a standard iodine or 2,6-dichlorophenolindophenol solution and then used in inoculating *N. glutinosa* plants. The titration values were used to calculate the amount of ascorbic acid which had become oxidized over a definite period of time. From these data curves for the rate of oxidation of ascorbic acid were plotted. The average numbers of lesions per half leaf of *N. glutinosa* plants produced by the aliquots of the reaction mixtures were used as a criterion of the degree of infectivity. By plotting these numbers of lesions, expressed

in per cent of the number of lesions produced on the opposite halves by the control solutions, against the time of reaction, curves were obtained which represented the rate of inactivation of the virus in the reaction mixture.

Effect of dialysis. An observed loss of infectivity in the virus-ascorbic acid systems, as determined by inoculation tests, could conceivably be due either to fundamental changes in the nature of the virus or to some effect of ascorbic acid on the plant which might prevent the plant from producing the characteristic symptoms of infection. Virus preparations previously inactivated in virus-ascorbic acid systems were dialyzed against redistilled water until free from ascorbic acid and dehydroascorbic acid, as indicated by chemical tests. The results of inoculation of such dialyzed material to *N. glutinosa* plants showed that the virus did not regain its ability to produce necrotic lesions on this host. These results indicate that ascorbic acid under suitable conditions produces a fundamental change in the nature of the virus. This change manifests itself by the loss of infectivity of the virus.

EFFECTIVE CONCENTRATIONS OF REACTANTS

The inactivation reaction was studied with various concentrations of ascorbic acid. It is apparent from the results shown in Table I that under the conditions of the experiment as previously described, inactivation of the virus occurred at all concentrations of ascorbic acid from the highest concentration used in these experiments, 20 mg. per cc., to 0.010 mg. per cc. The length of time necessary for the completion of the inactivation reaction increased with the diminution of the concentration of ascorbic

TABLE I

INACTIVATION OF TOBACCO MOSAIC VIRUS AS INFLUENCED BY THE TIME OF REACTION AND BY THE CONCENTRATION OF ASCORBIC ACID
CONCENTRATION Cu^{++}, 10^{-4} MG./CC.

Conc. of ascorbic acid mg./cc.	Inactivation*									
	1 Min.	3 Min.	5 Min.	7 Min.	10 Min.	15 Min.	30 Min.	1 Hr.	3 Hrs.	24 Hrs.
20.000	+ −		+	+	+	+				
5.000	−	+ −	+	+	+	+	+			
1.000			+ −	+	+	+	+	+		
0.150			−	−	+ −	+	+	+	+	+
0.050					−	+ −	+	+	+	+
0.030					−	+ −	+	+	+	+
0.025						−	+ −	+	+	+
0.020						−	+ −	+ −	+	+
0.015						−	−	+ −	+	+
0.010						−	−	+ −	+	+
0.005						−	−	−	−	+ −
0.002						−	−	−	−	−

* + = complete inactivation; + − = partial inactivation; − = no activation.

acid in the virus-ascorbic acid systems. The inactivation occurred in a few minutes at the higher concentrations of ascorbic acid, while at the lower concentrations the reaction required several hours. Under the conditions of the experiment, no complete inactivation of the virus in the virus-ascorbic acid systems resulted at concentrations below 0.010 mg. of ascorbic acid per cc.; at a concentration of 0.005 mg. per cc. partial inactivation occurred over a period of 24 hours, while at a concentration of 0.002 mg. per cc. there were no signs of loss of infectivity of the virus in the reaction mixtures after 24 hours.

EFFECT OF OXIDATION-REDUCTION POTENTIALS

In view of the fact that ascorbic acid is a reducing substance, the question naturally arises whether the inactivation of the virus in virus-ascorbic acid systems was produced by a direct oxidation-reduction reaction between the ascorbic acid and the virus. If this were the case, evidently other reducing substances possessing oxidation-reduction potentials of the same value or lower than the oxidation-reduction potential of ascorbic acid might also be able to inactivate the virus. The effects of several other reducing substances on the infectivity of the virus were tested. The oxidation-reduction potentials of ascorbic acid, cysteine and glutathione were calculated by the formulae[2] suggested by Green (8, 9). This investigator reported that the values he obtained by the colorimetric method for the oxidation-reduction potentials of these substances differed from the values he obtained by the potentiometric titration method. In view of the differences in the values of the oxidation-reduction potentials as calculated by the two methods, the concentrations and the pH values for the solutions of cysteine and of glutathione were adjusted to produce oxidation-reduction potentials corresponding in one case to the colorimetric, and in the other case to the potentiometric values of the oxidation-reduction potentials of ascorbic acid. This necessitated having the reaction mixtures at different pH values (pH 4.4 to pH 7.1). At both these oxidation-reduction potentials cysteine and glutathione failed to inactivate the virus under the conditions of the experiment, while ascorbic acid under similar conditions produced complete inactivation of the virus (Table II.) At more negative oxidation-reduction potentials, which were obtained by bringing the pH values of the media to pH 7 and to pH 8 without changing any of the other conditions of the experiment, cysteine and glutathione also failed to inactivate the virus over a period of 24 hours.

[2] For ascorbic acid $E_h = 0.375 - 0.060$ pH. Colorimetric 40 m.v. more negative than potentiometric.

For cysteine $E_h = 0.030 - 0.060$ pH $- 0.060 \log [\mathrm{CySH}]$. Colorimetric 90 m.v. more positive than potentiometric.

For glutathione $E_h = 0.062 - 0.060$ pH $- 0.060 \log [\mathrm{GSH}]$. Colorimetric 60 m.v. more positive than potentiometric.

The effects of hydroquinone and pyrogallol on the infectivity of the virus were also investigated. It was found that at pH 7 and at pH 8 hydroquinone at concentrations as low as 0.03 mg. per cc. could produce complete inactivation of the virus over a period of 24 hours, provided traces of cupric ions were present in the virus-hydroquinone systems. Pyrogallol under similar conditions and also at higher concentrations had no inactivating properties. These observations are of special interest in view of the findings of Bezssonoff and coworkers (5) that the oxidation-reduction potential of pyrogallol is lower than that of hydroquinone. These results,

TABLE II

EFFECT OF ASCORBIC ACID, CYSTEINE, AND GLUTATHIONE AT SIMILAR OXIDATION-REDUCTION POTENTIALS ON THE INFECTIVITY OF TOBACCO MOSAIC VIRUS CONCENTRATION Cu^{++}, 10^{-4} MG./CC.

Reducing substance	Concentration		pH	Oxidation-reduction potential* calculated according to		Inactivation in 24 hours
	Mg./cc.	Molar		Potentiometric determinations	Colorimetric determinations	
Ascorbic acid	0.171	0.0010	7.14	−0.053	—	Complete
"	0.037	0.0002	7.14	−0.053	—	"
"	0.171	0.0010	6.76	—	−0.071	"
"	0.037	0.0002	6.76	—	−0.071	"
Cysteine	0.121	0.0010	4.38	−0.053	—	None
"	0.024	0.0002	5.08	−0.053	—	"
"	0.121	0.0010	6.13	—	−0.068	"
"	0.024	0.0002	6.88	—	−0.071	"
Glutathione	0.216	0.0010	4.95	−0.055	—	"
"	0.043	0.0002	5.62	−0.053	—	"
"	0.216	0.0010	6.13	—	−0.066	"
"	0.043	0.0002	6.92	—	−0.071	"

* Calculated using formulae given by Green (8, 9).

as well as the observed stability of the virus in the presence of cysteine and of glutathione at oxidation-reduction potentials equal to the oxidation-reduction potentials of ascorbic acid, suggest that the inactivation of the virus in virus-ascorbic acid systems is not due to a simple oxidation-reduction reaction between these two substances in which ascorbic acid acts as a reducing agent.

EFFECT OF DIFFERENT GASES AND OXIDIZING AGENTS

Ascorbic acid-virus systems in ordinary distilled water, containing traces of cupric ions, were kept in atmospheres of each of the following gases: air, oxygen, nitrogen, carbon dioxide, hydrogen, and hydrogen sulphide. Aliquots were removed at intervals for titration with a standard iodine solution and for tests of infectivity. The results (Table III) indicate that in the atmosphere of oxygen or of air the virus in the reaction mixture gradually loses its infectivity and the ascorbic acid becomes reversibly

TABLE III

EFFECT OF DIFFERENT GASES ON THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY ASCORBIC ACID CONCENTRATION Cu^{++}, 10^{-4} MG./CC.

Concn. ascorbic acid, mg./cc.	Duration of reaction Hrs.	Duration of reaction Min.	Gas bubbled through soln.	No. of spots, expressed in % of spots produced by untreated soln.	Condition of ascorbic acid at end of reaction
0.050	0	40	Air	2, av. dev. 2	Oxidized form
0.168	2	0	"	0	"
0.050	4	30	"	0	"
0.050	24	0	"	0	"
0.050	0	40	O_2	0	"
0.050	2	0	"	0	"
0.050	0	40	N_2	76, av. dev. 22	Reduced form
0.254	1	0	"	94 " " 26	"
0.170	1	0	"	85 " " 23	"
0.050	4	30	"	108 " " 16	"
0.050	24	0	"	97 " " 21	"
0.030	24	0	"	95 " " 16	"
0.050	96	0	"	78 " " 20	"
0.168	2	0	H_2	90 " " 17	"
0.168	2	0	CO_2	124 " " 30	"
0.050	0	40	H_2S	104 " " 15	"
0.050	24	0	"	120 " " 42	"
0.050	96	0	"	118 " " 11	"

oxidized. Under similar conditions in atmospheres of any of the other gases tested, the virus retained its infectivity throughout the experimental period, and the ascorbic acid remained in the reduced form. Treatment of the ascorbic acid in the reaction mixture with oxidizing agents other than oxygen, such as iodine, 2,6-dichlorophenolindophenol, or potassium permanganate, resulted in oxidation of ascorbic acid which was not accompanied by inactivation of the virus. It follows from these results that inactivation of tobacco mosaic virus by ascorbic acid requires the presence of oxygen, and that this inactivation is accompanied by autoxidation of the ascorbic acid in the reaction mixture.

EFFECT OF DIFFERENT pH VALUES ON THE INACTIVATION REACTION

The rate of oxidation of ascorbic acid and the rate of inactivation of the virus were determined in reaction mixtures which were adjusted to various pH values (pH 3 to pH 8) by suitable Sörensen's buffers. The relative infectivity of each reaction mixture was tested against control virus suspensions adjusted to the respective hydrogen ion activities of the ascorbic acid-virus systems. The results obtained are summarized in Table IV and Figure 1. It can be seen that the rate of oxidation of ascorbic acid and the rate of inactivation of the virus are both at their highest values in approximately neutral reaction media, and decrease with either increase or decrease in acidity. However, complete inactivation of the virus was asso-

TABLE IV

EFFECT OF pH VALUES ON THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY ASCORBIC ACID

CONCENTRATION ASCORBIC ACID, 1.1×10^{-1} MG./CC.
CONCENTRATION Cu^{++}, 10^{-4} MG./CC.

pH	Buffer	Ascorbic acid oxidized at inactivation, mg./cc.	Time required for inactivation, minutes
3	Phthalate	0.093*	637*
4	"	0.063	124
5	"	0.057	42
6	"	0.036	15.5
7	Phosphate	0.034	6
8	Borate	0.019	16

* Partial inactivation.

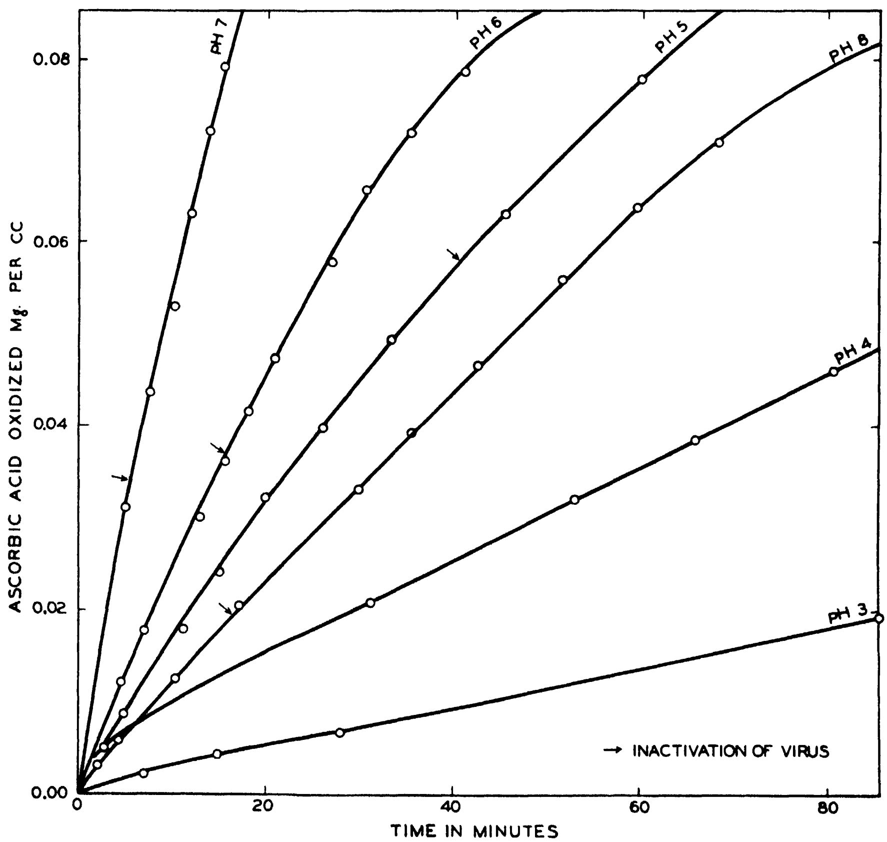


FIGURE 1. Rate of autoxidation of ascorbic acid and inactivation of virus in virus-ascorbic acid systems at different pH values. Concentrations Cu^{++}, 10^{-4} mg. per cc.

ciated with oxidation of progressively larger quantities of ascorbic acid as the acidity increased (Table IV). At pH 3 ascorbic acid failed to produce complete inactivation of the virus during a period of 10½ hours, in spite of the fact that about 85 per cent of the ascorbic acid originally present in the reaction mixture became oxidized during that period. The solubility of the crystalline virus material in slightly acid media has been reported (43) to decrease with increased acidity. It appears possible that the resistance of the virus to inactivation by ascorbic acid increases with decrease in solubility as the pH value of the virus-ascorbic acid systems approaches the isoelectric point (pH 3.3) of the crystalline protein (24, 36).

EFFECT OF DEHYDROASCORBIC ACID ON THE INFECTIVITY OF THE VIRUS

Dehydroascorbic acid obtained by the oxidation of ascorbic acid with iodine and by autoxidation of ascorbic acid in the presence of hexoxidase invariably failed to inactivate the virus in virus-dehydroascorbic acid systems both in the presence and in the absence of copper. Dehydroascorbic acid obtained by the autoxidation of ascorbic acid under the catalytic effect of cupric ions was able to inactivate the virus over periods of 24 hours. The experiments were performed at pH 6 and a concentration of dehydroascorbic acid of 0.11 mg. per cc. As in the case of ascorbic acid the inactivation proceeded only in the presence of oxygen. Removal of oxygen from the reaction mixture by bubbling nitrogen through the solution inhibited the inactivation reaction. As compared with the reaction in the ascorbic acid-virus systems, the inactivation reaction in the dehydroascorbic acid-virus systems proceeded at a much lower velocity.

The nature of the reaction involved in the inactivation of the virus in dehydroascorbic acid-virus systems has not been studied. However, the results obtained from these experiments are of great importance, as they definitely indicate that dehydroascorbic acid does not act directly on the virus to produce its inactivation. The low velocity of the inactivation reaction in the dehydroascorbic acid-virus systems, as well as the failure of dehydroascorbic acid to inactivate the virus in the absence of oxygen, exclude the possibility that the inactivation of the virus in ascorbic acid-virus systems is produced by the dehydroascorbic acid formed from the oxidation of ascorbic acid.

CATALYSTS

Effect of cupric ions. Solutions free of traces of copper ions were prepared by using water freshly redistilled from a quartz still and by dialyzing the virus preparations against such water. It can be seen from the data represented in Figure 2 that in such solutions the virus can remain in contact with ascorbic acid in the presence of oxygen without losing its infectivity. No oxidation of ascorbic acid occurs under these conditions.

Small measured amounts of cupric chloride were added to copper-free solutions and the rate of oxidation of ascorbic acid and the rate of inactivation of the virus were determined. The results of such determinations are shown in Figure 3 for solutions containing cupric ions in a concentration of

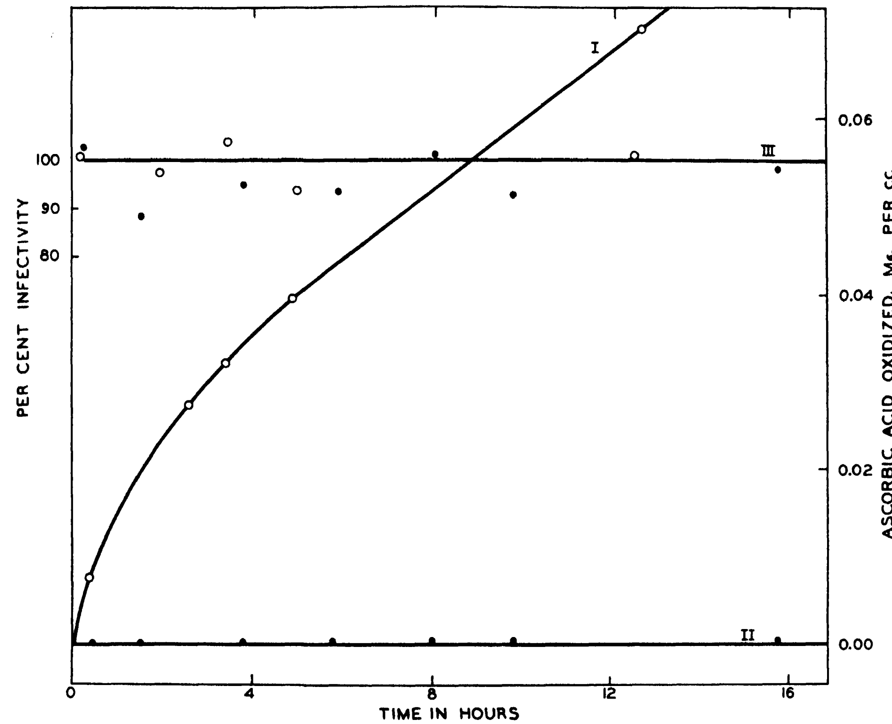


FIGURE 2. Rate of autoxidation of ascorbic acid and corresponding infectivity of tobacco mosaic virus in catalyst-free virus-ascorbic acid systems at pH 8.6 and at pH 6.0. I. Autoxidation of ascorbic acid at pH 8.6. II. Autoxidation of ascorbic acid at pH 6. III. Infectivity of virus at pH 8.6 and at pH 6.

10^{-4} and of 0.5×10^{-4} mg. per cc. From these curves it can be seen that while ascorbic acid is being oxidized by oxygen under the catalytic effect of cupric ions, the virus in the solution loses its infectivity. In the absence of ascorbic acid, cupric ions at the concentrations used had no effect on the infectivity of the virus. By comparing the results of the two sets of experiments in which the concentration of cupric ions in the reaction mixture is the only factor of difference, one can see that both the rate of oxidation of ascorbic acid and the rate of inactivation of the virus increase with increased concentrations of cupric ions.

Effect of alkalinity. The results represented in Figure 2 show that when the autoxidation of ascorbic acid is induced by bringing the pH of the

solution to 8.6 in the absence of copper, complete oxidation of ascorbic acid can occur without resulting in measurable inactivation of the virus. It follows from these observations that there must be a difference in the process of autoxidation of ascorbic acid catalyzed on the one hand by

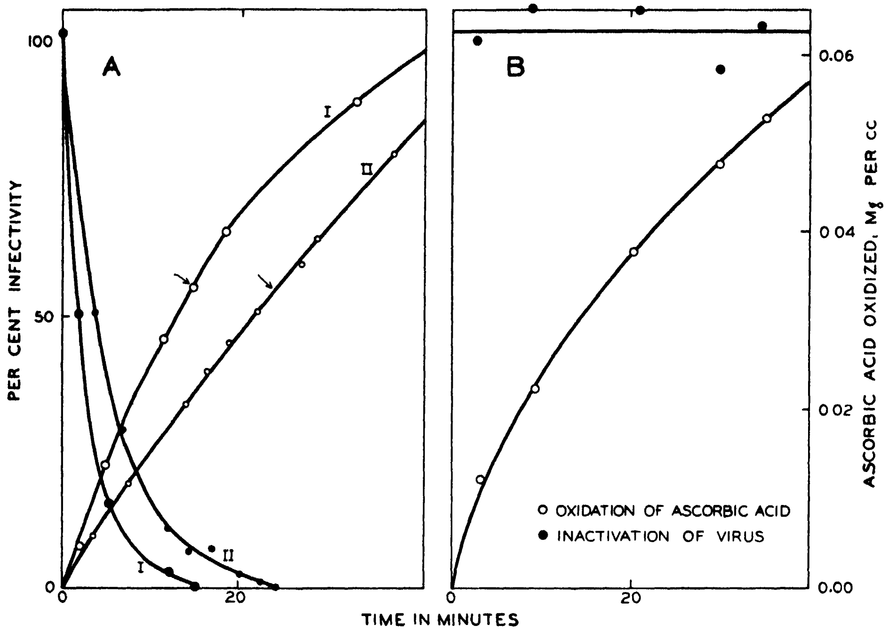


FIGURE 3. Rate of autoxidation of ascorbic acid and corresponding infectivity of tobacco mosaic virus in the presence of catalysts in the virus-ascorbic acid systems. A. Cupric ions used as catalyst: I. Concentration Cu^{++}, 10^{-4} mg. per cc. II. Concentration Cu^{++}, 0.5×10^{-4} mg. per cc. B. Hexoxidase used as catalyst.

cupric ions and on the other by the alkalinity of the media, since the former is accompanied by inactivation of the virus, while the latter does not affect the infectivity of the virus.

Effect of hexoxidase. Another catalyst, the enzyme hexoxidase, catalyzes the autoxidation of ascorbic acid (39). It was found that this autoxidation was not accompanied by loss of the infectivity of the virus in the reaction mixture. The enzyme was prepared from freshly picked leaves of young cabbage (*Brassica oleracea* L.) plants by a method similar to the one used by Szent-Györgyi (39). This method consisted essentially in the removal of inert substances from extracted cabbage juice by precipitation with barium acetate, and the precipitation of active substances by saturated ammonium sulphate, followed by solution with a phosphate buffer at pH 6. It was found that this hexoxidase preparation at a concentration of one per cent had a catalytic activity approximately equal to that

of cupric ions at a concentration of 10^{-4} mg. per cc. (Figure 3). In order to make certain that the catalytic effect of the hexoxidase was not complicated by any catalytic effects of traces of metallic ions which might be present in the solution, a sample of the enzyme preparation was heated to boiling and its activity was tested. The boiled enzyme completely failed to catalyze the autoxidation of ascorbic acid. Figure 3 indicates that when the oxidation of ascorbic acid proceeded at the same rate under the catalytic effect of cupric ions and hexoxidase, respectively, the autoxidation catalyzed by hexoxidase in the absence of copper had no effect on the infectivity of the virus, while autoxidation catalyzed by cupric ions resulted in the inactivation of the virus.

These results suggest that the reversible autoxidation of ascorbic acid can proceed in at least two different ways. The difference between these two processes can be detected by following the effect of the autoxidation on the infectivity of tobacco mosaic virus in ascorbic acid-virus systems. The autoxidation of ascorbic acid in alkaline media as well as the autoxidation catalyzed by the enzyme hexoxidase proceeds without affecting the infectivity of the virus, while autoxidation catalyzed by traces of cupric ions results in inactivation of the virus in the reaction mixture. The results suggest that the inactivation of the virus is due to the formation of some intermediate product in the process of autoxidation of ascorbic acid under the catalytic effect of cupric ions.

INACTIVATION OF THE VIRUS IN UNPURIFIED PLANT JUICE

Ascorbic acid did not produce inactivation of the virus in the crude juice of diseased Turkish tobacco plants under the conditions resulting in the inactivation of the crystalline virus. The unpurified juice was used at a concentration matching in infectivity with the purified virus suspensions. In the presence of healthy tobacco juice in the reaction mixture containing the usual concentration of crystalline virus material, together with ascorbic acid and cupric ions, the inactivation of the virus also failed to occur. The results represented in Table V indicate that this inhibiting effect could be partially overcome by increasing the concentration of cupric ions in the reaction mixtures. Consequently some of the constituents of the plant juice responsible for this inhibiting effect evidently belong to the class of substances which remove ionic copper from the reaction mixture. These results are in agreement with the observation of other investigators (3, 20, 25) that the inhibiting effect of tissue extracts and of some other substances is due to the ability of these agents to remove ionic copper and prevent it from catalyzing the autoxidation of ascorbic acid.

The inhibiting effect of the diluted plant juice could also be reduced by increasing the concentration of ascorbic acid in the reaction mixtures or by heating the juice to a temperature of 60° C. This latter observation sug-

TABLE V

INACTIVATION OF TOBACCO MOSAIC VIRUS BY ASCORBIC ACID IN THE PRESENCE OF CRUDE TOBACCO PLANT JUICE
DURATION OF REACTION, 24 HOURS

Ascorbic acid mg./cc.	Cu^{++} concentration mg./cc.	Inactivation*				
		Crystalline virus			Virus in crude diseased juice	
		Pure	Added healthy juice			
			Not heated	Heated 60° C. 30 m.	Not heated	Heated 60° C. 30 m.
0.00	10^{-4}	−	−	−	−	−
0.10	"	+	−	−	−	−
0.25	"	+	−	−	−	−
0.50	"	+	−	−	−	−
1.00	"	+	−	−	−	−
5.00	"	+	−	−	−	−
0.00	2.5×10^{-3}	−	−	−	−	−
0.10	"	+	+−	+	+−	+
0.25	"	+	+−	+	+−	+
0.50	"	+	+	+	+	+
1.00	"	+	+	+	+	+
5.00	"	+	+	+	+	+

* + = complete inactivation; +− = partial inactivation; − = no inactivation.

gests that the inhibiting effect could also be produced by some heat labile substances, possibly of the nature of enzymes.

EFFECT OF CATALASE ON THE INACTIVATION REACTION

Many autoxidation reactions have been reported (27) to proceed with the formation of peroxides as intermediate products. Although most of these intermediate peroxides are very unstable, in some cases as in the autoxidation of benzaldehyde the peroxide has been isolated. It has been found in the course of this investigation that benzaldehyde at a 0.003 molar concentration was able to produce inactivation of the virus over a period of 72 hours.

It has been suggested that an intermediate product is formed in the process of autoxidation of ascorbic acid. Experiments were undertaken with the purpose of determining whether this intermediate product is a peroxide. Color tests for detection of hydrogen peroxide (chromic acid test) and for organic peroxides (guaiacum and benzidine tests) were applied to solutions of ascorbic acid which were undergoing autoxidation under the catalytic effect of cupric ions. The results were negative. These results do not necessarily indicate the absence of peroxide in the solution, since ascorbic acid by virtue of its reducing properties could be expected to interfere with this reaction. Moreover, the velocity of the decomposition

of the peroxide might be the factor which prevented the development of the characteristic colors. The interfering action of ascorbic acid in this type of tests was confirmed by the observation that dilute solutions of

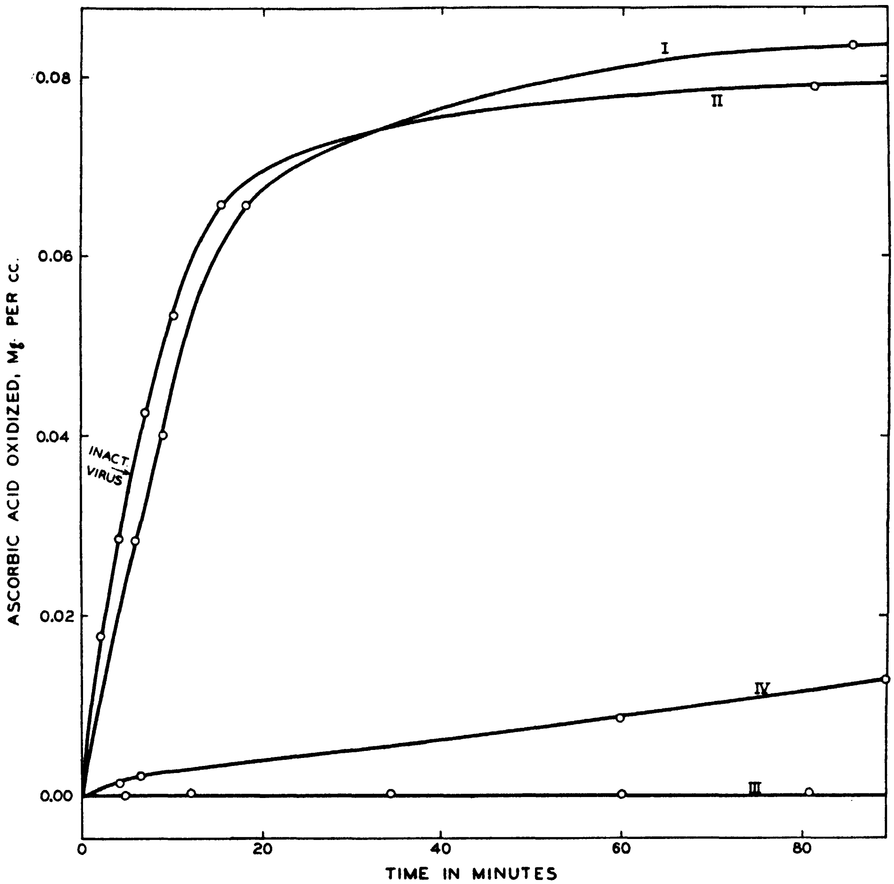


FIGURE 4. Effect of catalase on the rate of autoxidation of ascorbic acid and on the inactivation of tobacco mosaic virus in virus-ascorbic acid systems. I. Concentration Cu^{++}, 5×10^{-4} mg. per cc.; catalase present. II. Concentration Cu^{++}, 5×10^{-4} mg. per cc.; inactivated catalase present. III. No Cu^{++}; catalase present. IV. Concentration Cu^{++}, 10^{-4}; catalase present.

hydrogen peroxide failed to give a positive chromic acid test in the presence of ascorbic acid.

If the inactivating properties of ascorbic acid were due to the formation of a peroxide as an intermediate product in the process of autoxidation of ascorbic acid, evidently a reagent which could destroy the peroxide as soon as it was formed in the virus-ascorbic acid system would inhibit the

inactivation of the virus. Catalase is specific in its ability to decompose peroxides without liberating active oxygen (30). This enzyme attacks both organic and inorganic peroxides (37). A crude preparation of catalase was obtained from cow's liver by Stern and Battelli's method (38, p. 1373–1382). Preliminary tests indicated that this enzyme was very potent in its ability to decompose hydrogen peroxide. Small amounts of the catalase preparation were introduced into ascorbic acid-virus systems which contained cupric ions at a concentration 5×10^{-4} mg. per cc. (At a concentration of 10^{-4} mg. per cc. of cupric ions the catalase preparation inhibited the autoxidation of ascorbic acid.) The changes occurring in the systems were followed by determining the amount of ascorbic acid oxidized and of the virus inactivated in the reaction mixture. It was observed that the ascorbic acid was rapidly undergoing autoxidation, while the virus in the reaction mixture retained its infectivity (Figure 4). As controls, similar experiments were performed in systems containing a catalase preparation inactivated by previous heating at 60° C. for 30 minutes. The heated preparation possessed no catalase activity, as indicated by its failure to decompose hydrogen peroxide. The results obtained in the two systems, one of which contained active and the other inactivated catalase preparations, are represented in Figure 4. While the autoxidation of ascorbic acid in the two systems proceeded with the same velocity, the autoxidation was accompanied by complete inactivation of the virus in the system containing inactivated catalase, but no inactivation of the virus occurred in the presence of active catalase in the virus-ascorbic acid systems. These results were confirmed several times under slightly different conditions of pH, temperature, and concentration of reactants. They strongly support the theory that the inactivation of the tobacco mosaic virus in virus-ascorbic acid systems is produced by a peroxide which is formed as an intermediate product in the autoxidation of ascorbic acid under the catalytic effect of copper.

DETERMINATION OF THE NATURE OF THE ACTIVE PEROXIDE

The inhibiting effect of catalase on the inactivation of virus in ascorbic acid-virus systems was considered as strong evidence of the formation of an active peroxide in the course of this reaction. An attempt was made to determine the nature of the peroxide. There appeared to be three possibilities: the peroxide might be hydrogen peroxide, as suggested by Barron and coworkers (2); a copper peroxide; or an organic peroxide.

Hydrogen peroxide. Allard (1) and later Stanley (32) reported that hydrogen peroxide at concentrations of three per cent and of one per cent, respectively, reduced the infectivity of the virus. Similar results were obtained in this investigation at concentrations of the order of 0.02 per cent. Colloidal platinum is known to catalyze the decomposition of hydro-

gen peroxide (6). An investigation of the effect of colloidal platinum on the inactivation of the virus in virus-hydrogen peroxide, and in virus-ascorbic acid systems revealed that the presence of this catalyst inhibited the inactivation reaction in the former system by decomposing the hydrogen peroxide in the system. The presence of colloidal platinum in the latter system did not inhibit the inactivation reaction. If hydrogen peroxide were the active peroxide causing inactivation of the virus in virus-ascorbic acid systems, the presence of colloidal platinum would evidently have the same effect on the inactivation reaction in both systems.

According to the theory of Barron and coworkers (2), one molecule of hydrogen peroxide is produced as an intermediate product by the autoxidation of each molecule of ascorbic acid. In this study quantitative determinations revealed that in virus-hydrogen peroxide systems, at concentrations of hydrogen peroxide theoretically equivalent to the minimum inactivating concentrations of ascorbic acid, inactivation of the virus did not occur. An eight-fold increase over the concentration of hydrogen peroxide resulted in inactivation of the virus over a period of 24 hours.

Since the introduction of colloidal platinum into the ascorbic acid-virus system did not prevent the inactivation of the virus, and the introduction of even eight-fold the theoretical ascorbic acid equivalent of hydrogen peroxide promoted inactivation of the virus only at a very slow rate, it would seem that hydrogen peroxide was not the responsible agent for the inactivation of the virus.

Copper peroxide. The very important rôle played by cupric ions in the inactivation reaction suggested that the active substance might conceivably be a copper peroxide. The reddish-brown copper peroxide having the theoretical formula $CuO_2 \cdot H_2O$ (26, p. 116) was prepared by the following method: cupric hydroxide was stirred with an excess of dilute hydrogen peroxide at a temperature of 5° C.; a trace of ferrous hydroxide was added to catalyze the reaction; when the mixture had reached the reddish-brown color typical for copper peroxide, it was filtered by suction and the precipitate was washed with ice cold water, until the washings were free from hydrogen peroxide (chromic acid test). The washed precipitate was treated with alcohol and with ether and dried in vacuo. The copper peroxide obtained by this method gave a positive chromic acid test. Infectivity tests showed that the virus did not lose its infectivity in virus-copper peroxide systems which contained copper peroxide at concentrations higher than the concentrations theoretically equivalent to the inactivating concentrations of ascorbic acid.

The inhibiting effect of catalase on the inactivation of tobacco mosaic virus in virus-ascorbic acid systems indicated that the inactivation was produced by an active peroxide formed as an intermediate product in the autoxidation of ascorbic acid. Neither hydrogen peroxide nor cupric per-

oxide could explain the inactivation reaction. It appears therefore that the active substance is an ascorbic acid peroxide, or possibly a copper ascorbate peroxide.

DISCUSSION

In the course of this work it was found that ascorbic acid was able to produce complete inactivation of the tobacco mosaic virus. A study of the conditions under which this inactivation occurs revealed that the inactivation proceeded only when the ascorbic acid in the reaction mixture was undergoing autoxidation under the catalytic effect of cupric ions. These results together with the reported ability of tobacco mosaic virus to undergo inactivation under the influence of oxidizing agents (32) suggest that this inactivation reaction in virus-ascorbic acid systems belongs to the class of induced oxidation reactions. This theory is supported by the results of a recent investigation of Holtz (15), who reported that in sugar-ascorbic acid systems the oxidation of ascorbic acid by oxygen was accompanied by oxidation of sugar at a greatly increased rate as compared with the oxidation in control ascorbic acid-free sugar solutions. Holtz concluded from these observations that ascorbic acid acts as an inductor in the oxidation of sugar.

In the present work it was observed that the virus can remain in contact with the reduced form of ascorbic acid indefinitely without becoming inactivated, provided the ascorbic acid is not undergoing autoxidation. The same was true for the dehydroascorbic acid. Evidently neither the ascorbic acid nor the dehydroascorbic acid can react directly with the virus and cause its inactivation. If, however, the ascorbic acid undergoes autoxidation under the catalytic effect of cupric ions and changes from the reduced to the oxidized form, the virus in the solution loses its infectivity. These observations suggest that the inactivation of the virus is produced by an intermediate product formed during the autoxidation of ascorbic acid. The further observation that inactivation does not occur when the autoxidation proceeds in the presence of catalase indicates that the intermediate product is a peroxide. This supports to a certain extent the theory postulated by Barron and coworkers (2, 3) that a peroxide is formed as an intermediate product of the autoxidation of ascorbic acid with copper as catalyst. However, the difference in the rate of the inactivation reactions, as well as the difference in the concentrations of the reactants necessary for the inactivation reaction in ascorbic acid-virus and in hydrogen peroxide-virus systems, respectively, suggest that the active substance is a peroxide other than hydrogen peroxide. Cupric peroxide also failed to explain the inactivation reaction, thus suggesting that the active substance is probably an organic peroxide. The formation of organic peroxides as intermediate products has been reported to occur in other autoxidation and induced oxidation reactions (27).

From the study of the inactivating effect of the autoxidation of ascorbic acid in slightly acid media in the presence of cupric ions, on the one hand, and in the presence of the enzyme hexoxidase on the other, it was concluded that the reversible autoxidation of ascorbic acid can proceed in at least two ways. While one type of autoxidation has no effect on the infectivity of the virus present in the reaction mixture, the other type, which is catalyzed by cupric ions, results in the inactivation of the virus in virus-ascorbic acid systems.

In spite of the very important rôle which ascorbic acid plays in the animal body and probably also in the plants, its mode of action in the living organism has not yet been determined. The theory that ascorbic acid plays an important part in the cell respiration has been postulated by several investigators (4, 13, 29). The finding that ascorbic acid acts as an inductor in the reaction resulting in the inactivation of tobacco mosaic virus suggests the possibility that this vitamin might have a similar function in living organisms. While undergoing autoxidation in the living body ascorbic acid might conceivably induce the oxidation of some substances which in the absence of ascorbic acid would not be oxidized by oxygen. Consequently in the absence of ascorbic acid in the living cells a disturbance in the normal oxidation processes of the body could occur, and might be followed by a development of pathological conditions.

SUMMARY

Autoxidation of ascorbic acid under the influence of cupric ions is associated with a capacity to inactivate highly purified tobacco mosaic virus in ascorbic acid-virus systems.

The autoxidation of ascorbic acid which occurs in an alkaline medium or in the presence of the catalyst, hexoxidase, is not accompanied by the capacity to inactivate the virus.

The inactivation of tobacco mosaic virus in the presence of ascorbic acid undergoing reversible oxidation catalyzed by cupric ions is attributable to the formation of a specific intermediate product in the course of the autoxidation of the ascorbic acid. Neither ascorbic acid nor dehydroascorbic acid is capable of reacting directly with the virus to effect its inactivation.

The inactivation of the virus by autoxidation of ascorbic acid in the presence of cupric ions is inhibited by catalase, thus indicating that the intermediate product responsible for the inactivation is a peroxide.

ACKNOWLEDGMENTS

The author is indebted to Dr. Lela E. Booher for her helpful advice and criticism, and to N. S. Lojkin for his helpful suggestions and assistance in performing the experiments. She also wishes to express her thanks to Dr. Helen Purdy Beale and Dr. Frank Wilcoxon for their valuable suggestions, and to Dr. William Crocker, Director of the Boyce Thompson

Institute for Plant Research, Inc., for granting her the permission to perform this investigation as a research problem of Boyce Thompson Institute.

LITERATURE CITED

1. ALLARD, H. A. Some properties of the virus of the mosaic disease of tobacco. Jour. Agric. Res. **6**: 649–674. 1916.
2. BARRON, E. S. GUZMAN, R. H. DEMEIO, and FRIEDRICH KLEMPERER. Studies on biological oxidations. V. Copper and hemochromogens as catalysts for the oxidation of ascorbic acid. The mechanism of the oxidation. Jour. Biol. Chem. **112**: 625–640. 1936.
3. BARRON, E. S. GUZMAN, ALBERTO GUZMAN BARRON, and FRIEDRICH KLEMPERER. Studies on biological oxidations. VII. The oxidation of ascorbic acid in biological fluids. Jour. Biol. Chem. **116**: 563–573. 1936.
4. BESSEY, OTTO A., and C. G. KING. The distribution of vitamin C in plant and animal tissues, and its determination. Jour. Biol. Chem. **103**: 687–698. 1933.
5. BEZSSONOFF, N., A. DELIRE, et H. VAN WIEN. L'activité réductrice de la vitamine C et d'autres réducteurs biologiques exprimée en fonction de leur concentration. Bull. Soc. Chim. Biol. **16**: 1133–1159. 1934.
6. BREDIG, G., und R. MÜLLER VON BERNECK. Über anorganische Fermente. I. Über Platinkatalyse und die chemische Dynamik des Wasserstoffsuperoxyds. Zeitschr. Physik. Chem. **31**: 258–353. 1899.
7. GAGYI, J. v. Über die bakterizide und antitoxische Wirkung des Vitamin C. Klin. Wochenschr. **15**: 190–195. 1936. (*Abstr. in* Zentralbl. Bakt. Abt. 2. **95**: 74. 1936.)
8. GREEN, DAVID EZRA. The reduction potentials of cysteine, glutathione and glycylcysteine. Biochem. Jour. **27**: 678–689. 1933.
9. ——— The potentials of ascorbic acid. Biochem. Jour. **27**: 1044–1048. 1933.
10. GREENWALD, CHARLES K., and E. HARDE. Vitamin C and diphtheria toxin. Proc. Soc. Exp. Biol. & Med. **32**: 1157–1160. 1935.
11. GROOTEN, O., et N. BEZSSONOFF. La sensibilité du bacille de la coqueluche vis-a-vis de la vitamine C et de l'hydroquinol. Compt. Rend. Soc. Biol. **120**: 121–123. 1935.
12. HARDE, EDNA. Acide ascorbique (vitamine C) et intoxications. Compt. Rend. Acad. Sci. [Paris] **199**: 618–620. 1934.
13. HARRISON, DOUGLAS CREESE. The action of vitamin C on the oxidation of tissues in vitro. Biochem. Jour. **27**: 1501–1505. 1933.
14. HOLMES, FRANCIS O. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929. (*Also in* Contrib. Boyce Thompson Inst. **1**: 504–520. 1929.)
15. HOLTZ, PETER. Über die Induktionswirkung der Ascorbinsäur auf die Oxydation der Zucker. Arch. Exp. Path. Pharmakol. **182**: 109–111. 1936.
16. JOHNSON, SYDNEY WALGATE. The regeneration of the reducing properties of oxidized lemon juice. Biochem. Jour. **27**: 1287–1289. 1933.
17. JUNGEBLUT, CLAUS W. Inactivation of poliomyelitis virus in vitro by crystalline vitamin C (ascorbic acid). Jour. Exp. Med. **62**: 517–521. 1935.
18. ——— Vitamin C therapy and prophylaxis in experimental poliomyelitis. Jour. Exp. Med. **65**: 127–146. 1937.
19. JUNGEBLUT, CLAUS W., and RAYMUND L. ZWEMER. Inactivation of diphtheria toxin in vivo and in vitro by crystalline vitamin C (ascorbic acid). Proc. Soc. Exp. Biol. & Med. **32**: 1229–1234. 1935.
20. KELLIE, ALEXANDER EDWARDS, and SYLVESTER SOLOMON ZILVA. The catalytic oxidation of ascorbic acid. Biochem. Jour. **29**: 1028–1035. 1935.
21. KLIGLER, I. J. Inhibitive effect of vitamin C on toxin production by *C. diphtheria.* Nature [London] **138**: 291. 1936.

22. LOJKIN, MARY. Inactivation of tobacco mosaic virus by ascorbic acid. Contrib. Boyce Thompson Inst. **8**: 335. 1936.
23. LOMINSKI, IWO. Inactivation du bactériophage par l'acide ascorbique. Compt. Rend. Soc. Biol. **122**: 766–768. 1936.
24. LORING, HUBERT S., and W. M. STANLEY. Isolation of crystalline tobacco mosaic virus protein from tobacco plants. Jour. Biol. Chem. **117**: 733–754. 1937.
25. MAWSON, COLIN ASHLEY. The influence of animal tissues on the oxidation of ascorbic acid. Biochem. Jour. **29**: 569–579. 1935.
26. MELLOR, J. W. A comprehensive treatise on inorganic and theoretical chemistry. Vol. 3. Longmans, Green and Co. London. 1923.
27. MILAS, NICHOLAS A. Auto-oxidation. Chem. Reviews **10**: 295–364. 1932.
28. POLÓNYI, PAUL VON. Einfluss des C-Vitamins auf den Krankheitserreger der Diphtherie. Vorläufige Mitt. Wiener Med. Wochenschr. **1935**: 685.
29. QUASTEL, JUDAS HIRSCH, and ARNOLD HERBERT MAURICE WHEATLEY. An effect of ascorbic acid on fatty acid oxidations in the liver. Biochem. Jour. **28**: 1014–1027. 1934.
30. ROBERTSON, T. BRAILSFORD. Principles of biochemistry. 2nd ed. 796 pp. Lea & Febiger, Philadelphia. 1924.
31. SAMUEL, GEOFFREY, and J. G. BALD. On the use of the primary lesions in quantitative work with two plant viruses. Ann. App. Biol. **20**: 70–99. 1933.
32. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. IV. Some effects of different chemical agents on infectivity. Phytopath. **25**: 899–921. 1935.
33. ——— Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. Science **81**: 644–645. 1935.
34. ——— Chemical studies on the virus of tobacco mosaic. VI. The isolation from diseased Turkish tobacco plants of a crystalline protein possessing the properties of tobacco-mosaic virus. Phytopath. **26**: 305–320. 1936.
35. ——— Chemical studies on the virus of tobacco mosaic. VII. An improved method for the preparation of crystalline tobacco mosaic virus protein. Jour. Biol. Chem. **115**: 673–678. 1936.
36. ——— Chemical studies on the virus of tobacco mosaic. VIII. The isolation of a crystalline protein possessing the properties of aucuba mosaic virus. Jour. Biol. Chem. **117**: 325–340. 1937.
37. STERN, KURT G. On the mechanism of enzyme action. A study of the decomposition of monoethyl hydrogen peroxide by catalase and of the intermediate enzyme-substrate compound. Jour. Biol. Chem. **114**: 473–494. 1936.
38. STERN, LINA, und FRÉDERIQ BATTELLI. Katalase. *In* Oppenheimer, Carl. Die Fermente und ihre Wirkungen. 5 Aufl., Bd. 3. 1578 pp. Georg Thieme, Leipzig. 1929.
39. SZENT-GYÖRGYI, ALBERT. On the function of hexuronic acid in the respiration of the cabbage leaf. Jour. Biol. Chem. **90**: 385–393. 1931.
40. TILLMANS, J., P. HIRSCH, und H. DICK. Das Reduktionvermögen pflanzlicher Lebensmittel und seine Beziehung zum Vitamin C. IV. Über die Reversibilitat der Oxydationen des reduzierenden Stoffes im Citronsaft. Zeitschr. Untersuch. Lebensm. **63**: 267–275. 1932.
41. VINSON, C. G. The virus diseases of plants. *In* Missouri Agric. Exp. Sta. Bull. 358. 84. 1935.
42. ——— Virus diseases of plants. Purification of the virus of mosaic disease of tobacco. Missouri Agric. Exp. Sta. Res. Bull. 237. 16 pp. 1936.
43. WYCKOFF, RALPH W. G., J. BISCOE, and W. M. STANLEY. An ultracentrifugal analysis of the crystalline virus proteins isolated from plants diseased with different strains of tobacco mosaic virus. Jour. Biol. Chem. **117**: 57–71. 1937.

PREPARATION OF PLANT GROWTH-PROMOTING SUBSTANCES. I. 1-NAPHTHALENEGLYOXALIC ETHYL ESTER; 1-NAPHTHALENEGLYCOLLIC ACID; 1-NAPHTHALENEACETIC ACID

Frank Wilcoxon

The two possible naphthalene-substituted acetic acids have been described in the chemical literature several times in the past fifty years. The 1-acid has been known since 1883 when it was first prepared by Boessneck (4), while the 2-acid was obtained thirteen years later by Blank (3). In 1887 Willgerodt (12) obtained the amide of the 1-acid by heating naphthylmethyl ketone with sulphur and yellow ammonium sulphide in a sealed tube for several days at a temperature of 210° to 230°. For many years interest in these compounds was slight, but the recent discovery that 1-naphthaleneacetic acid is an effective growth-promoting substance leads to renewed interest in the methods available for synthesis of these acids (15). The original method of Boessneck involved the following series of reactions: 1-naphthoyl chloride→1-naphthoyl cyanide→1-naphthoylformamide→1-naphthoylformic acid. On reduction of the ketone acid by means of red phosphorus and hydriodic acid, 1-naphthaleneacetic acid was obtained. It was described as easily soluble in hot water, but slightly soluble in the cold, and crystallized in fine, long, silky, odorless needles which melted at 131° C. In 1908, Tiffeneau and Daudel (11) obtained the 1-acid from 1-naphthylethanal by oxidation with silver hydroxide in an alkaline medium. Mayer and Oppenheimer (9) in 1914 described the preparation of both the naphthaleneacetic acids by the bromination of methylnaphthalene. The product of bromination was converted to the cyanide by aqueous-alcoholic sodium cyanide, and the nitrile thus obtained hydrolyzed to give the corresponding naphthaleneacetic acid. The series of transformations may be represented as follows: 1-naphthylmethyl bromide→1-naphthylmethyl cyanide→1-naphthylacetic acid. The yield was stated to be 49 per cent of the theoretical.

In the same year Wislicenus and Elvert (13) obtained the ethyl esters of the naphthaleneacetic acids by a similar series of reactions, except that the nitrile was converted directly to the ethyl ester. A yield of 36 per cent based on the 1-methylnaphthalene was claimed for the corresponding ester. Mauthner (8), in 1917, applied his general method for the synthesis of aryl-substituted fatty acids to the preparation of 1-naphthaleneacetic acid. This method consists in condensing an aromatic aldehyde with hippuric acid to give an azlactone, which on hydrolysis gives ammonia, benzoic acid, and the corresponding substituted pyruvic acid. The latter is treated with hydrogen peroxide, forming the desired fatty acid by elimination of carbon dioxide.

In 1929, Gilman and Kirby (5) prepared the two naphthaleneacetic acids by means of the Grignard reaction, from the naphthylmethyl chlorides. The latter compounds were prepared from the naphthylmethylcarbinols, since the products obtained by direct chlorination of methylnaphthalene gave unsatisfactory results in the subsequent Grignard reaction. The entire series of reactions may be represented as follows: 1-bromonaphthalene→1-naphthylmagnesium bromide→1-naphthylmethyl carbinol → 1-naphthylmethyl chloride → 1-naphthaleneacetic acid. The yield based on 1-bromonaphthalene was 24 per cent of the theoretical.

In 1933, Keach (7) obtained 1-naphthaleneacetic acid by oxidation of 1-allylnaphthalene with potassium permanganate. The melting point of his product was 106° C., while the accepted melting point is 131°. In the following year Higginbottom and Short (6) made a study of Keach's method and succeeded in purifying the reaction product, obtaining the acid melting at 132° C.

One of the most recent preparations of naphthaleneacetic acid is that of Arndt and Eistert (2). These authors have recently (1935) described a general method for transforming carboxylic acids into their higher homologues, or into derivatives of the latter. This method involves treatment of the acid chloride with diazomethane solution, forming a diazoketone. The latter when treated with water, ammonia, or an amine in the presence of a catalyst such as finely divided silver, copper, or platinum undergoes a rearrangement, splitting off nitrogen, and taking up the elements of water, ammonia, or the amine, leading to a homologue of the original acid used

$$R \cdot CO \cdot CHN_2 + H \cdot OH \longrightarrow R \cdot CH_2 \cdot COOH + N_2$$

The yield in the case of naphthaleneacetic acid was about 32 per cent of theory.

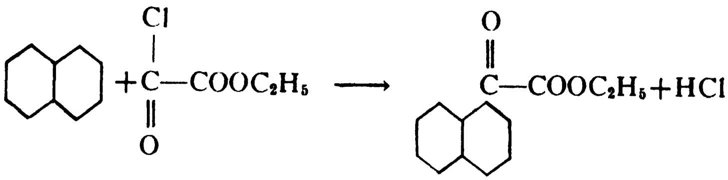
In spite of the numerous descriptions of the preparation of naphthaleneacetic acid in the literature, there is little information on its properties, or those of its derivatives. Many of the methods of synthesis described are troublesome, or require as starting materials substances not readily available. For these reasons it was decided to prepare the acid and study its properties, making use of a method which lends itself to the preparation of considerable quantities, which requires readily accessible raw materials, and which results in fairly good yields.

The method of Mayer and Oppenheimer, which involves the bromination of 1-methylnaphthalene, led, in our hands, to a product very difficult to purify. This may have been due in part to the use of impure 1-methylnaphthalene.

U. S. Patent No. 1,951,686 to Wolfram, Schörnig, and Hausdörfer (14)

describes the preparation of naphthaleneacetic acid by heating naphthalene and chloroacetic acid together. We were unable to obtain any of the acid by following the procedure disclosed in the patent.

Rousset (10) has prepared the ethyl ester of naphthaleneglyoxalic acid by the Friedel and Crafts reaction using naphthalene and chloroglyoxalic ethyl ester.



A yield of the α- and β-ketone esters of 50 per cent of the theoretical was obtained.

It was decided to study this method since reduction of the ketone acid would yield either naphthaleneacetic acid or naphthaleneglycollic acid, depending upon the method of reduction used, while naphthaleneglyoxalic acid would be obtained by saponification. The latter two acids are of interest as possible growth hormones and have not yet been tested by workers in this field.

PREPARATION OF 1-NAPHTHALENEGLYOXALIC ETHYL ESTER

The method of Rousset was modified in several details. A typical experiment was performed as follows: 29.1 g. of naphthalene (0.2276 mols.) was dissolved in 109 cc. (1.064 mols.) of nitrobenzene, and the solution cooled to 0° C. in an ice bath. Forty g. of anhydrous aluminum chloride (0.3000 mols. $AlCl_3$), were added gradually while the solution was being stirred mechanically. After the addition of the aluminum chloride, 19.887 g. or 0.1456 mols. of chloroglyoxalic ethyl ester, obtained as described by Adickes, Brunnert, and Lücker (1) were added drop by drop. The evolution of HCl appeared to be complete after about five hours. The solution was treated with cracked ice and steam distilled until most of the nitrobenzene was removed. The product was separated and fractionated in vacuo. A yield of 15.74 g. or 47.4 per cent of the theoretical was obtained. The product was almost entirely the desired α-isomer, since conversion to the picrate and fractional crystallization of the latter gave only the picrate of the 1-ester, melting at 77° C. (10).

REDUCTION OF 1-NAPHTHALENEGLYOXALIC ETHYL ESTER

(a) *By sodium amalgam.* The ester (2.38 g.) was saponified by heating with sodium hydroxide. The solution was adjusted to slight alkalinity with HCl and NaOH. It was then allowed to stand in contact with sodium amalgam overnight. On filtration and acidification 1-naphthaleneglycollic acid was precipitated in rosettes of needles. Recrystallized from hot water

it melted at 98° C. The yield was 40 per cent of the theoretical. Microtitration gave values for the molecular weight of 200 and 201. The calculated value for $C_{12}H_{10}O_3$ is 202.08. Microchemical combustion gave the following results. 1-naphthaleneglycollic acid. Calc. for $C_{12}H_{10}O_3$: C, 71.25; H, 4.99. Found: C, 71.31, 71.39; H, 4.89, 5.15.

FIGURE 1. 1-naphthaleneacetic acid recrystallized from hot water.

(b) *Catalytic reduction with nickel catalyst.*[1] The ester (5.017 g.) dissolved in alcohol was reduced with hydrogen in the presence of Raney nickel catalyst. The reduction proceeded fairly rapidly at atmospheric pressure and room temperature and from the reduced product 1-naphthaleneglycollic acid was obtained in 88 per cent yield.

[1] The experimental work on this topic was performed by Mr. Julian Riesenberg of the Polytechnic Institute, Brooklyn, New York.

(c) *Reduction with hydriodic acid and red phosphorus.* The ester (2.343 g.) was boiled for three hours with a mixture of 1.5 g. of red phosphorus and 18 g. of hydriodic acid (sp. gr. 1.7). The reaction mixture was made alkaline with sodium hydroxide, filtered, and the 1-naphthaleneacetic acid precipitated with HCl. The yield of crude product was 90 per cent of the theoretical. After several recrystallizations from hot water the acid melted at 131° C. Microtitration gave values for the molecular weight of 185.5 and 186. The calculated value is 186.08. Microchemical combustion gave the following results. 1-naphthaleneacetic acid. Calc. for $C_{12}H_{10}O_2$: C, 77.38; H, 5.41. Found: C, 77.35, 77.42; H, 5.19, 5.23. The acid on recrystallization from hot water was obtained in fine long needles, the characteristic appearance of which is illustrated in Figure 1.

The material as obtained from hot water was submitted to Prof. C. W. Mason of Cornell University for microscopic examination, who reports as follows: "The crystals as received are very thin lath-shaped rectangular plates, edge and flat views of which give parallel extinction. Since the acute bisectrix is apparently perpendicular to the plane of these plates, they are in all probability orthorhombic.

"Viewed flatwise they give a good biaxial positive interference figure, with the axial plane lengthwise of the crystals. 2 E is about 30°. Dispersion of the optic axes is small, $r > v$. Maximum birefringence is shown by plates on edge, with γ (vibrations crosswise) about 1.75, and α (vibrations lengthwise) 1.605. Plates lying flatwise show α (vibrations lengthwise), and β (vibrations crosswise) 1.620."

Solubility determinations were made by agitating an excess of the acid with water in a thermostatic bath held at 20.5° C. for 24 hours. By evaporation of filtered samples to dryness, two duplicate determinations gave 41 mg. and 42 mg. per 100 cc. at 20.5° C.

All these compounds, 1-naphthaleneglyoxalic ethyl ester, 1-naphthaleneglycollic acid, and 1-naphthaleneacetic acid exhibit the characteristic properties of plant growth-promoting substances in varying degrees. A more detailed study of their physiological effects will be presented elsewhere.

SUMMARY

Methods of preparation of 1-naphthaleneacetic acid have been briefly reviewed. The acid has been prepared by the following series of reactions: condensation of naphthalene with chloroglyoxalic ethyl ester to give 1-naphthaleneglyoxalic ethyl ester with a yield of about 50 per cent; reduction of the latter with hydriodic acid and red phosphorus to give 1-naphthaleneacetic acid. The solubility of 1-naphthaleneacetic acid in water at 20.5° C. has been found to lie between 41 and 42 mg. per 100 cc.

LITERATURE CITED

1. ADICKES, F., W. BRUNNERT, und O. LÜCKER. Über die Herstellung einiger Säureester. Jour. Prakt. Chem. **130**: 163–176. 1931.
2. ARNDT, F., und B. EISTERT. Ein Verfahren zur Überführung von Carbonsäuren in ihre höheren Homologen bzw. deren Derivate. Ber. Deutsch. Chem. Ges. **68**: 200–208. 1935.
3. BLANK, OSCAR. Notiz über β-Naphthylessigsäure. Ber. Deutsch. Chem. Ges. **29**: 2373–2375. 1896.
4. BOESSNECK, P. Derivate der α-Naphtoesäure. Ber. Deutsch. Chem. Ges. **16**: 639–642. 1883.
5. GILMAN, HENRY, and JAMES E. KIRBY. Some rearrangement reactions of alpha-naphthylmethylmagnesium chloride. Jour. Amer. Chem. Soc. **51**: 3475–3478. 1929.
6. HIGGINBOTTOM, ALICE, and W. F. SHORT. 1-naphthylacetic acid. Rec. Trav. Chim. Pays-Bas **53**: 1141. 1934.
7. KEACH, DEWITT T. The synthesis of α-naphthylacetic acid. Jour. Amer. Chem. Soc. **55**: 2974–2975. 1933.
8. MAUTHNER, F. Ueber eine allgemeine Synthese der Phenylfettsäuren. Justus Liebig's Ann. Chem. **370**: 368–375. 1909.
9. MAYER, FRITZ, und TRUDI OPPENHEIMER. Über Naphthyl-essigsäuren. (1. Abhandlung). Ber. Deutsch. Chem. Ges. **49**: 2137–2141. 1914.
10. ROUSSET, L. Action du chlorure d'éthoxalyle sur le naphtalène et les éthers oxydes des naphtols en presence du chlorure d'aluminium. Bull. Soc. Chim. France, Sér. 3. **17**: 300–312. 1897.
11. TIFFENEAU et DAUDEL. Transposition phénylique. Migration du groupe naphtyle chez les iodhydrines de la série du naphtalène. Compt. Rend. Acad. Sci. [Paris] **147**: 678–680. 1908.
12. WILLGERODT, C. Ueber die Einwirkung von gelbem Schwefelammonium auf Ketone und Chinone. Ber. Deutsch. Chem. Ges. **20**: 2467–2470. 1887.
13. WISLICENUS, WILHELM, und HEINRICH ELVERT. Esterkondensationen mit Naphthylessigestern. Ber. Deutsch. Chem. Ges. **49**: 2820–2830. 1914.
14. WOLFRAM, ARTHUR, LUDWIG SCHÖRNIG, and EMIL HAUSDÖRFER. New process of preparing arylacetic acids and new product obtainable thereby. U. S. Patent No. 1,951,686. 1934.
15. ZIMMERMAN, P. W., and FRANK WILCOXON. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

A RETRIAL OF THE ETHYLENE CHLORHYDRIN METHOD FOR HASTENING THE GERMINATION OF FRESHLY-HARVESTED GLADIOLUS CORMS[1]

F. E. Denny

Subsequent to the recommendation (1) that dormant *Gladiolus* corms be treated with ethylene chlorhydrin in order to hasten germination, a number of reports of tests by others (3, 4, 5, 6) have been published. In all cases the comments have been more or less unfavorable to the method, and in no reported test of any variety was the germination hastened in a manner comparable to that reported in the previous paper (1).

A particularly unfavorable report is the most recent one by Gilbert and Pember (3) who described their experimental results as follows: (p. 9) "Finally local corms dug October 24, 1934, were treated on November 19 with varying concentrations of ethylene chlorhydrin vapor after being peeled. They were planted and the check untreated corms germinated before the treated ones with no benefit from the treatment in any way."

Since these results were so much at variance with those obtained in our previous tests it seemed advisable to retry the treatments to determine whether the favorable effect is obtainable only with the varieties previously tested.

Nine varieties not tried in the previous test were included in the experiments and these were treated not only at the period shortly after harvest but also after a subsequent cold-storage period for 3 weeks at 5° C. The germination of all nine varieties was hastened by the treatment with ethylene chlorhydrin, although with four of them the most favorable response was not obtained until after a cold-storage period had intervened before the application of the chemical. The number of days gained by the treatment of freshly-harvested and not cold-stored lots could not be computed for at least six of the varieties because the germination of the untreated corms was so low, being either zero or less than 5 per cent at the expiration of 6 months; but in the cases in which the controls germinated sufficiently well to furnish a numerical value the gain due to treatment was 3 to 5 months. With the cold-stored lots the gain due to chemical treatment depended upon the response of the control corms to the cold-storage period; with two varieties the gain was only 10 days, with four it was 50 days, and with three it was not computable because of low germination of the controls, but was at least greater than 4 months.

The incomplete germination response of four of these varieties when tested soon after harvest and previous to any preliminary cold-storage period may be the explanation of the unfavorable reports of others who

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 135.

may have experimented with bulbs in a similar condition of dormancy; but in the present tests the gain shown even in the least favorable test was sufficient to indicate the desirability of further tests with other varieties or with such varieties at other stages of the rest period.

METHODS

The varieties Mrs. Frank Pendleton, Mrs. F. C. Peters, and Dr. F. E. Bennett were harvested on September 24, 1936, and the other varieties were harvested October 13, 1936. After harvest the corms were spread loosely on the floor at room temperature and allowed to stand for 9 to 11 days before treatments were applied. The outer brown husks were not removed previous to treating the corms since previous experiments have shown that the results were practically the same whether or not the husks were removed. The corms were placed in vessels of one to two liters capacity in lots usually of 25 corms each. The required quantity of ethylene chlorhydrin (the commercial 40 per cent solution) was distributed throughout a piece of cheesecloth which was then spread loosely on a piece of filter paper resting on top of the corms to be treated. The vessel was then sealed and allowed to stand at room temperature for the required period, which in the present tests was 2 days for some of the lots and 4 days for others. The quantity of ethylene chlorhydrin was determined by the weight of the corms in the different lots and varied from 0.5 cc. to 2.0 cc. per 100 grams of corms. With all varieties the sizes of the corms were such that the weight per corm varied from 14 to 26 grams, except for Senorita, the corms of which were smaller, weighing only 9 grams each. As soon as the treatment ended the corms were planted in soil in flats which were stored at room temperature, and close attention was paid to proper and uniform watering of the soil. The germination record refers to emergence of sprouts from the soil in the flats.

Because of the previous results (2) showing the favorable effect of a period of low temperature storage upon germination, a portion of the supply of corms was placed in burlap bags and stored for 3 weeks in a constant temperature room regulated at 5° C. These cold-stored bulbs were then subjected to the same series of treatments as those applied in the period shortly after harvest.

RESULTS

TREATMENT OF FRESHLY-HARVESTED CORMS

The effect of the treatments upon the emergence of sprouts is shown in Table I. The results for treatments of freshly-harvested corms, i.e., at the 9th to 11th day after harvest, are shown in columns 4 to 9. Although the corms were so dormant that untreated corms did not show germination percentages greater than 28, and in some cases showed no sprouts at all

even after a period of 180 days from planting, good germination resulted from chemical treatments of the varieties Mrs. Frank Pendleton, Minuet, Scarlet Princeps, Senorita, and Mrs. Leon Douglas. With these, nearly complete germination was obtained before even one sprout appeared in the check lot. The treated bulbs of the varieties Minuet, Scarlet Princeps, and Mrs. Leon Douglas showed 90 to 100 per cent germination after 50 days, while the control lots showed 0 to 28 per cent germination after 180 days. The Senorita bulbs were much more dormant, possibly because of their smaller size, but distinct gains were shown, 84 to 88 per cent germination in the treated lots being obtained before any sprouts from the controls appeared.

With the varieties Mrs. F. C. Peters, Odin, and Dr. F. E. Bennett the treatments did not induce more than 48 per cent germination during the 6-month period, but it is seen that the controls during that period did not show more than 4 per cent germination.

TREATMENT OF CORMS AFTER COLD STORAGE

The effect of chemical treatment of corms which had been subjected to a 3-week period of storage at 5° C. before the chemical treatment was applied is shown in Table I, columns 10 to 15. It is seen that the varieties Mrs. F. C. Peters, Odin, and Dr. F. E. Bennett, whose germination had been only incompletely forced by treatments applied before the cold-storage period, responded satisfactorily to a treatment applied after cold storage, showing 92 to 100 per cent germination within 50 days from planting, while the percentage germination after 150 days for the controls (also cold-stored but not chemically treated) was 0 for Mrs. F. C. Peters, 60 for Odin, and 16 for Dr. F. E. Bennett. With Mrs. Frank Pendleton and Senorita, although the cold-storage treatment itself hastened the germination of the controls, a still further gain was obtained by the treatment so that, on the basis of time required for 50 per cent germination, the gain due to chemical treatment was 55 to 60 days. With Minuet, Scarlet Princeps, Mr. W. H. Phipps, and Mrs. Leon Douglas the cold-storage period had so effectively favored the germination of the corms that only 9 to 16 days additional gain was obtained by the treatment applied after cold storage.

Longer cold-storage periods were tested with all of the varieties except Dr. F. E. Bennett, 49 days at 5° C. with Minuet, Odin, Mrs. Leon Douglas, Scarlet Princeps, Senorita, and Mr. W. H. Phipps; 113 days at 5° C. with Mrs. Frank Pendleton and Mrs. F. C. Peters. Cold storage broke the rest period so well that, except for Senorita, the control lots germinated within 30 to 40 days and no gain due to treatment was obtained. With Senorita the treatment hastened the germination even under these conditions by about 50 days. But although the cold treatment had rendered the corms

of some of the varieties apparently entirely non-dormant the chemical treatments, even at the highest concentration needed for forcing, i.e., 1 cc. per 100 grams of corms for 4 days, did not retard the germination nor induce earlier germination of the controls as observed by Gilbert and Pember (3).

TREATMENT OF NON-GERMINATING CONTROLS

At the expiration of 128 days from harvest and 117 days from planting, when no sprouts had appeared from many of the non-treated lots, a portion of the planted corms were removed from the soil in the control lots of Mrs. Frank Pendleton and Mrs. F. C. Peters. These corms were dried in air at room temperature for 2 days, were then divided into lots, some of which were treated with ethylene chlorhydrin, while others were again planted as controls. The object was to see whether the corms were still viable, and whether chemical treatments could induce germination. At this stage the Mrs. Frank Pendleton corms were becoming less dormant and showed 50 per cent germination after 69 days without treatment. The treated lot, however, required only 28 days to reach this stage. The Mrs. F. C. Peters corms were still quite dormant, showing no sprouts after 100 days from time of planting, the treated lots giving 50 per cent germination after 24, 30, and 29 days, and 88, 100, and 100 per cent final germinations, respectively for the three treatments: 1 cc. per 100 grams for 4 days, 1 cc. for 2 days, and 0.5 cc. for 2 days.

CONDITION OF NON-GERMINATING CHEMICALLY-TREATED CORMS

At the end of the experiment the corms in the lots showing low germination in some or all of the treatments, e.g., Mrs. F. C. Peters, Mr. W. H. Phipps, Odin, and Dr. F. E. Bennett treated in the period shortly after harvest, were examined for corm injury. Only three lots showed evident injury, 16 per cent rot in the Mrs. F. C. Peters lot with 1 cc. per 100 grams for 4 days, 32 per cent in the Mr. W. H. Phipps lot with 2 cc. for 4 days, and 24 per cent in the Odin lot with 2 cc. for 4 days. In all other cases the recovered corms were sound, showing that the low germination was not due to injury, but to a failure to break the rest period.

Although in the three cases just described corm injury resulted from the treatment with these concentrations in the period previous to cold storage, it will be seen that these same concentrations when applied after the 3-week cold-storage period produced no injury, but brought about prompt germination. Cold storage had widened considerably the range of chemical concentration and period of exposure within which successful forcing could be accomplished. It would appear from the data in Table I, columns 10 to 15, that the range is much wider than that tested in these experiments.

TABLE I

EFFECT OF ETHYLENE CHLORHYDRIN UPON GERMINATION OF FRESHLY-HARVESTED GLADIOLUS CORMS

Variety	Chemical treatment		Corms not cold-stored Treatment applied at 9th to 11th day after harvest						Corms cold-stored Treatment applied after an additional 3-weeks' storage at 5° C.					
	Cc. per 100 g.	Days duration	Per cent germination, days from planting						Per cent germination, days from planting					
			40 da.	50 da.	70 da.	90 da.	130 da.	180 da.	30 da.	40 da.	50 da.	90 da.	110 da.	150 da.
Mrs. Frank Pendleton	1.0	4	73	76	76	76	76	80	23	73	91	100	100	100
	1.0	2	6	20	68	94	96	96	26	72	91	96	96	96
	0.5	2	0	20	54	71	100	100	13	70	90	100	100	100
	None	–	0	0	0	0	4	28	0	0	0	51	77	100
Mrs. F. C. Peters	1.0	4	25	44	48	48	48	48	40	87	100	100	100	100
	1.0	2	4	4	4	4	4	4	21	88	96	96	96	96
	0.5	2	0	0	0	0	0	0	26	68	92	96	96	96
	None	–	0	0	0	0	0	0	0	0	0	0	0	0
Minuet	2.0	4	28	58	86	96	96	96	30	86	96	96	96	96
	1.0	4	26	58	75	88	96	96	30	100	100	100	100	100
	1.0	2	42	92	100	100	100	100	47	100	100	100	100	100
	0.5	2	80	100	100	100	100	100	—	—	—	—	—	—
	None	–	0	0	0	0	0	24	0	8	64	100	100	100
Scarlet Princeps	2.0	4	65	78	80	80	80	80	69	98	100	100	100	100
	1.0	4	75	85	88	91	96	96	50	100	100	100	100	100
	1.0	2	44	73	77	80	80	88	50	100	100	100	100	100
	0.5	2	85	96	98	100	100	100	—	—	—	—	—	—
	None	–	0	0	0	0	0	4	0	55	84	100	100	100
Mr. W. H. Phipps	2.0	4	18	43	51	52	52	52	25	87	100	100	100	100
	1.0	4	24	38	48	48	48	48	46	77	88	92	92	92
	1.0	2	40	69	80	80	84	84	37	92	100	100	100	100
	None	–	0	0	0	0	24	28	0	36	52	72	76	76
Odin	2.0	4	11	16	16	16	16	16	85	100	100	100	100	100
	1.0	4	20	24	24	24	24	24	88	100	100	100	100	100
	1.0	2	19	38	44	44	44	44	80	80	80	80	80	80
	None	–	0	0	0	0	0	4	12	46	60	60	60	60
Senorita	1.0	4	0	6	29	49	56	60	6	11	72	100	100	100
	1.0	2	0	0	6	20	44	84	0	0	12	100	100	100
	0.5	2	0	0	29	44	64	88	0	0	20	100	100	100
	None	–	0	0	0	0	0	0	0	0	4	4	88	96
Mrs. Leon Douglas	1.0	4	61	83	90	92	92	92	100	100	100	100	100	100
	0.5	2	100	100	100	100	100	100	—	—	—	—	—	—
	None	–	0	0	0	0	0	0	21	65	88	100	100	100
Dr. F. E. Bennett	1.0	2	8	28	28	28	28	28	33	92	96	96	96	96
	0.5	2	16	40	40	40	40	40	56	91	92	92	92	92
	None	–	0	0	0	0	0	0	0	0	0	16	16	16

SUMMARY

Corms of nine varieties of gladiolus harvested in late September and early October were treated with vapors of ethylene chlorhydrin, some lots on the 9th to 11th day after harvest, and other lots after an additional storage period of three weeks at 5° C. The chemical treatment hastened the germination of all of the varieties in the test, but the most favorable results with four of the varieties were obtained only when the treatments were applied after the preliminary cold-storage period. With the varieties responding to treatment before cold storage the gain in time required for germination due to chemical treatment was 3 to 5 months, or even more in some cases, since the untreated corms failed to germinate at all within 6 months after planting. With the varieties needing a preliminary cold-storage period before the application of the chemical treatment the gain varied with different varieties from about 10 days to more than 4 months, depending upon the response of the untreated corms to the cold-storage period itself. Untreated corms of the variety Mrs. F. C. Peters remaining for 117 days in the soil without germinating were removed from the soil and treated with ethylene chlorhydrin, with the result that these treated corms germinated promptly and the controls remained dormant for at least another 100 days.

LITERATURE CITED

1. DENNY, F. E. Shortening the rest period of gladiolus by treatment with chemicals. Amer. Jour. Bot. **17**: 602–613. 1930. (*Also in* Contrib. Boyce Thompson Inst. **2**: 523–534. 1930.)
2. ———— Storage temperatures for shortening the rest period of gladiolus corms. Contrib. Boyce Thompson Inst. **8**: 137–140. 1936.
3. GILBERT, BASIL E., and FREDERICK R. PEMBER. Gladiolus culture with special reference to winter forcing. Rhode Island Agric. Exp. Sta. Bull. 255. 18 pp. 1935.
4. LOOMIS, W. E. Forcing gladiolus. Proc. Amer. Soc. Hort. Sci. **30** (1933): 585–588. 1934.
5. VOLZ, E. C. Storage of gladiolus corms. *In* Iowa Agric. Exp. Sta. Rept. **1933–34**: 152. 1934.
6. WEINARD, F. F., and S. W. DECKER. Experiments in forcing gladioli. Illinois Agric. Exp. Sta. Bull. 357: 343–361. 1930.

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN POTATO TUBERS[1]

LAWRENCE P. MILLER

When potato tubers (*Solanum tuberosum* L.) are treated with ethylene chlorhydrin under conditions which have been found to break the rest period (3, 4) appreciable quantities of the chemical are absorbed (5, 9). Studies have shown that subsequent to the end of the treatment period some chlorhydrin is released in the form of vapor from the tubers but a large proportion is decomposed within the tubers.

Tests of the stability of ethylene chlorhydrin in buffers at various pH values have shown that chlorhydrin is quite stable at the pH value of the treated tubers. Nevertheless its disappearance in treated tubers is rapid and in the present paper are presented data obtained in experiments in which the decomposition was studied as well as the subsequent distribution within the tubers of the chloride formed. These experiments have shown that cutting greatly facilitates the decomposition of the chlorhydrin and that subsequently there is considerable accumulation of chloride at the cut surface.

MATERIALS AND METHODS

Materials. The potatoes used for these experiments consisted of tubers of the Irish Cobbler and Bliss Triumph varieties grown in the Institute garden, as well as tubers of the Irish Cobbler variety obtained from South Carolina and from New Jersey. Some tests were also made with tubers of the Bliss Triumph variety obtained from Florida. Since no varietal differences were observed and since the potatoes obtained from the various sources responded in essentially the same manner, the variety and source of the tubers used in obtaining the data given in the tables are not designated.

Methods. Most of the experiments reported in the present paper were made on tubers after the peel had been removed. The tubers were exposed to the chemical in vapor form or were soaked in an aqueous solution. At the end of the treatment the tubers were thoroughly washed in tap water and rinsed with distilled water to remove any chlorhydrin that might adhere to the surface. Some of the material was immediately taken for analysis to determine the amount taken up. The remaining tubers were then put into a large desiccator which was placed in a thermostatically controlled water bath kept at 25.5° C. A stream of air was drawn through the desiccator in order to furnish oxygen for respiration and to remove the CO_2 formed as well as to remove any ethylene chlorhydrin that was

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 139.

being given off by the tubers. The air on leaving the desiccator was drawn through Van Slyke-Cullen tubes containing $Ba(OH)_2$ or KOH solutions which served to decompose the chlorhydrin. The chloride content of these solutions could subsequently be determined by titration. The air entering the desiccator was drawn through alkaline solutions to remove CO_2 and other substances which might be contained in the laboratory air.

By periodic sampling of the tissue and determination of the chlorhydrin content and determination of the chlorhydrin given off as vapor, the rate of decomposition of the chlorhydrin within the tubers could be followed.

The chlorhydrin present in the tubers was recovered by distillation, the distillate being collected in a flask containing $Ba(OH)_2$ which reacts with the chlorhydrin to release the chloride ion which can be titrated. Details of the method are given in a previous paper (9). It has been estimated that by this method 85 per cent of the chlorhydrin present is recovered.

The method used for the determination of the total chloride content was based on that of Sunderman and Williams (12). Larger samples were taken than by Sunderman and Williams and therefore larger amounts of the reagents were necessary. This method involves a preliminary alkaline digestion which is followed by an open Carius method and thus the chlorine in any unaltered chlorhydrin would also be included. The determinations were made either on tissue samples, aliquots of the expressed juice or on aliquots of an alcoholic extract of the tissue.

RESULTS

STABILITY OF ETHYLENE CHLORHYDRIN IN BUFFERS

Since ethylene chlorhydrin is decomposed rather rapidly in treated potato tubers, it is important to know whether this decomposition is brought about by the metabolic activities of the tubers or whether it is merely due to the natural instability of chlorhydrin. Ethylene chlorhydrin is not a perfectly stable substance and aqueous solutions always contain some free hydrochloric acid. However, after the formation of a small amount of hydrochloric acid, decomposition does not proceed further. Such solutions usually have a pH value of from 3.5 to 4.0 and are apparently very stable under laboratory conditions. As would be expected the presence of alkalies hastens the splitting off of HCl. Some work has been done on the stability of ethylene chlorhydrin on boiling (1, 2) and in the presence of N/50 KOH and of more concentrated alkalies (6, 10) but no reports seem to have been published showing the behavior of chlorhydrin under conditions more closely approaching those found in the potato. Therefore some experiments were conducted in which chlorhydrin was added to potato juice and to buffers at various pH values and the amount of decomposition determined after various intervals. The data in Tables I

and II show that chlorhydrin is relatively stable in expressed potato juice and in buffers below about pH 7.5. Although treatments with ethylene chlorhydrin increase the pH of potatoes (7, 8), the pH values obtained in

TABLE I

STABILITY OF ETHYLENE CHLORHYDRIN IN POTATO JUICE*

Days from start of test	Cc. 0.1 molar chlorhydrin recovered
0	26.0
3	25.4
6	25.7
14	25.3
82	24.4
306	22.4

* 10 cc. of 40% ethylene chlorhydrin were added to 850 cc. expressed juice, the volume made up to one liter, toluene added and the mixture stored at room temperature. For analysis 50 cc. portions were taken, 275 cc. water added and 200 cc. distillate collected and the chlorhydrin determined.

TABLE II

DECOMPOSITION OF ETHYLENE CHLORHYDRIN AT ROOM TEMPERATURE IN BUFFERS AT VARIOUS pH VALUES

Buffer used	pH	pH at end of test	Duration of tests, months	Cc. 0.1 molar free chloride per 100 cc. at end*	% chlorhydrin decomposed
Phosphate	5.31	5.04	10	0.65	2.8
	5.54	5.48		0.35	1.6
	5.92	5.87		0.40	1.8
	6.41	6.34		1.15	5.3
	6.93	6.90		1.70	7.8
	7.66	7.30		4.45	20.4
Water control	4.16	3.51		0.25	1.2
Phosphate and citric acid	6.95	6.88	9	1.80	8.3
Phosphate	7.12	7.03		1.95	8.9
Phosphate	7.86	7.39		4.25	19.5
Phosphate and citric acid	7.86	7.59		6.8	31.2
Sodium borate	9.2**	—		21.4	100
Sodium carbonate and	10**	—		21.9	100
Sodium borate	11**	—		21.75	100

* Chlorhydrin present at start was equivalent to 21.8 cc. molar solution per 100 cc. of mixture.

** Theoretical pH values of buffer mixtures used; pH not determined.

the expressed juice, which are probably a little higher than those in the potato because of the escape of CO_2, are seldom more than 7.0, and more usually between 6.6 and 6.8, and no values as high as 7.5 have been obtained. The relatively rapid decomposition of chlorhydrin in treated potatoes must therefore be associated with the metabolic activity of the tubers.

DECOMPOSITION OF CHLORHYDRIN IN VARIOUS PORTIONS OF THE TUBERS

In experiments in which the chlorhydrin content of the treated tubers was to be followed from day to day, it was found advisable to use tubers with the peel removed since a much more uniform absorption of chlorhydrin could be obtained in this way. Previous experiments (9) have shown that with whole tubers the permeability of the peel is a very important factor in determining the amount of chlorhydrin taken up. Determination of the chlorhydrin content of individual tubers after being exposed to chlorhydrin vapor under the same conditions has indicated that the amount of chlorhydrin taken up when whole tubers of the same lot are exposed to the vapor varies considerably from tuber to tuber (unpublished results). On the other hand, when peeled tubers are exposed to chlorhydrin vapor or soaked in aqueous solutions of the chemical, the amount taken up by the individual tubers is quite uniform. The rate of decomposition can then be followed by periodic analyses of samples of the treated tissues.

To determine the distribution of chlorhydrin in the tubers successive layers were peeled from the tubers and the chlorhydrin content of these layers determined. This was done with tubers that had been soaked in aqueous solutions of chlorhydrin and with tubers that had been exposed to chlorhydrin vapor. The quantity of tissue removed at each peeling was regulated in such a way that the weight of each of the four layers removed was approximately the same. As a result each layer would therefore represent a portion thinner than the succeeding layer. The chlorhydrin distribution immediately after treatment as well as during the course of its decomposition could be readily followed in this way.

The results of a typical test in which the tubers were exposed to chlorhydrin vapor are shown in Table III and an experiment in which the tubers were soaked in a solution of chlorhydrin is given in Table IV. For the vapor treatment, 730 grams of potatoes, weighing about 23 grams each, were exposed to the vapor from 7 cc. of the 40 per cent solution for 17.5 hours, and in the soak treatment tubers of about the same size were immersed for one hour in a solution containing 40 cc. of 40 per cent ethylene chlorhydrin per liter. Immediately after the end of the treatment periods the tubers were washed and dried with cheesecloth, some of the tubers peeled into four layers of approximately the same weight and the chlorhydrin content determined.

To study the rate of disappearance of the absorbed chlorhydrin, analyses were also made on the various portions of the tubers at subsequent intervals as indicated in the tables. The amount of chlorhydrin lost as vapor in the intervals between the sampling periods was determined as described in the section headed "Methods." Since it was impossible to separate the tubers into four layers of exactly the same weight the figures shown in column 6 of Tables III and IV are not merely averages

of the values in the four preceding columns, but are corrected for differences in the actual weight of the four portions.

TABLE III

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN TREATED POTATOES (PEELED) (VAPOR TREATMENT)

Hours after end of treatment	Chlorhydrin present, cc. 0.1 M per 100 g.					Chlorhydrin lost during interval, cc. 0.1 M per 100 g. per hour	
	Part of tubers				Whole sample*	By decomposition	As vapor
	Outer	Second	Third	Inner			
0	47.6	50.7	42.4	26.0	42.5	—	—
23.8	36.0	38.2	39.7	36.3	37.7	0.17	0.03
51.8	19.5	23.0	24.1	22.1	22.3	0.52	0.02
75.5	13.0	16.3	17.5	17.9	16.4	0.19	0.05
95.5	8.2	9.2	10.6	10.7	9.6	0.23	0.11

* Corrected for small differences in weight of the four layers.

TABLE IV

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN TREATED POTATOES (SOAK TREATMENT)

Hours after end of treatment	Chlorhydrin present, cc. 0.1 M per 100 g.					Chlorhydrin lost during interval, cc. 0.1 M per 100 g. per hr.	
	Part of tubers				Whole sample*	By decomposition	As vapor
	Outer	Second	Third	Inner			
0	68.4	27.7	6.0	0.5	32.8	—	—
24	30.0	32.0	31.4	29.0	30.6	0.06	0.03
48	22.2	23.2	24.1	23.8	23.3	0.25	0.05
72	15.0	16.4	15.8	15.9	15.5	0.27	0.05
118	Trace	Trace	Trace	1.4	0.4	0.30	0.03

* Corrected for small differences in weight of the four layers.

As would be expected, in the soak treatment at the end of the one-hour period a large proportion of the chlorhydrin which had been absorbed was found in the outer layer, less in the next layer, and little to none in the innermost portion. But after 24 hours the chlorhydrin had become evenly distributed throughout. With the vapor treatment, in which an exposure period of 17 hours was employed, the absorbed chlorhydrin was more nearly equally distributed at the end of treatment, although the center portion contained less than the outer three. Even distribution was noted at the next sampling period, however. Analyses at subsequent intervals showed that the chlorhydrin content decreased rapidly and the chlorhydrin remaining was about evenly distributed throughout the tuber. Comparison of the values in the last two columns of Tables III and IV

shows that the amount of chlorhydrin given off as vapor averages less than one-fifth of the amount decomposed in the tubers. The results obtained when the distribution of the absorbed chlorhydrin was determined at the end of a soak treatment, and again 24 hours later, show that chlorhydrin becomes evenly distributed throughout the tubers rather quickly. The fact that the various layers show the same chlorhydrin content as the chlorhydrin is being decomposed therefore does not necessarily mean that the chlorhydrin is being decomposed at the same rate in all parts of the treated tubers. It is conceivable that the chlorhydrin is decomposed more rapidly in some portion of the tubers and that the undecomposed chemical is continually shifted so that its concentration remains about the same in all parts of the tubers. The evidence on the effect of cutting on the decrease in chlorhydrin content and the distribution of the chlorides in the tubers after the decomposition of the absorbed chemical indicates that more rapid decomposition takes place at the cut surface.

EFFECT OF CUTTING ON THE DECOMPOSITION OF THE ABSORBED CHLORHYDRIN

Analyses of planted seed pieces from treated tubers after sprouts appeared above ground (8 to 20 days after planting) showed that not more than a trace of the chlorhydrin remained in the sprouting seed pieces. Examination of the sprouts showed that they also did not contain any chlorhydrin. This indicated that the disappearance of the chlorhydrin was very rapid in the cut pieces which were planted for the sprouting test. Some comparisons were made of the chlorhydrin content of planted pieces which showed sprouts above ground and whole tubers from the same treatment which had been stored in air at room temperature in the interval. Such data are shown in Table V. The whole tubers still contained

TABLE V

DATA SHOWING THE MORE RAPID DECREASE IN ETHYLENE CHLORHYDRIN CONTENT OF PLANTED SEED PIECES IN CONTRAST TO WHOLE TUBERS STORED IN PAPER BAGS

Chlorhydrin recovered, cc. 0.1 molar per 100 grams			
Whole tubers			Corresponding planted pieces
At end of treatment	At a later period		
	Days	Chlorhydrin	
46	9	34	0.2
4		3	Trace
160		100	Trace
97	19	36	"
16		13	"

more than half of the absorbed chlorhydrin at the time of the test while sprouted seed pieces from the same treatment retained only a trace.

This result might be due to a leaching out of the chlorhydrin from the seed pieces into the soil in which they were planted or to a more rapid decomposition of the chlorhydrin in the planted pieces than in the whole tubers. In fact the experiments reported in the present paper show a more rapid decomposition of the chlorhydrin with the peeled tubers used than was formerly found for whole tubers. With the intact tubers, as shown in a previous paper (5, p. 177, Table XIII) the rate of decomposition of the absorbed chlorhydrin was only equivalent to from about 0.04 to 0.12 cc. of the 0.1 molar solution (0.31 to 0.93 mg.) per 100 grams per hour while the rate with the various lots of cut and peeled material used in the present experiments was considerably higher.

The effect of cutting could of course also be determined directly by experiment. In one experiment 1135 grams of peeled tubers weighing about 28 grams each were exposed to the vapor from 10 cc. of 40 per cent ethylene chlorhydrin for 18 hours and one-half of the tubers were stored as such and the other half further cut into pieces weighing about 7 grams each. Determinations were made of the amount of chlorhydrin present after various intervals, as well as of the amount of chlorhydrin given off as vapor, and the results are given in Table VI. The decrease in the chlorhydrin content was more rapid in the sliced tubers and this was due to a more rapid decomposition within the tubers and not to increased loss in the vapor form. In fact the quantity given off as vapor was greater in the whole tubers than in those cut into slices.

TABLE VI

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN TREATED TUBERS (PEELED TUBERS, VAPOR TREATMENTS)

Hours after end of treatment	Chlorhydrin, cc. 0.1 molar per 100 g. tissue			
	Stored as peeled tubers		Cut into slices at end of treatment	
	Chlorhydrin present	Chlorhydrin lost as vapor	Chlorhydrin present	Chlorhydrin lost as vapor
0	31.0	—	31.0	—
24.5	21.6	1.12	19.5	0.94
50.6	25.3	1.69	13.7	1.08
96.0	12.5	1.81	4.3	1.04
173.5	4.2	2.26	0.3	0.07

Results of another experiment on the effect of cutting on the rate of decomposition are shown in the first part of Table VII. In this case the tubers were cut into pieces weighing 20.6 grams and 2.6 grams each respectively, before treatment and the treatments were made by the soak method. In an attempt to obtain approximately equal concentrations in

the two lots, the larger pieces were soaked for 45 minutes in a solution containing 20 cc. of 40 per cent chlorhydrin per liter and the smaller pieces were soaked for 30 minutes in a solution containing 10 cc. of chlorhydrin per liter. As a result, at the start of the test the larger pieces contained the equivalent of 10.8 cc. 0.1 molar solution per 100 g. and the smaller pieces 7.9 cc. The chlorhydrin content decreased considerably more rapidly in the smaller pieces.

TABLE VII

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN TREATED TUBERS AND SUBSEQUENT CHLORIDE DISTRIBUTION WITHIN THE TUBERS

Hours from end of treatment	Chlorhydrin, cc. 0.1 molar per 100 grams			
	Pieces weighing 20.6 g.		Pieces weighing 2.6 g.	
	Chlorhydrin present	Lost as vapor during interval	Chlorhydrin present	Lost as vapor during interval
0	10.8		7.9	
20.5	9.6	0.4	5.9	0.2
44.0	5.9	0.3	1.1	0.1
88.3	Trace	0.2	Trace	0.1

Hours from end of treatment	Cc. 0.1 molar chloride per 100 grams						
	Treated or control	Large pieces			Small pieces		
		First layer	Rest of piece	Whole sample*	First layer	Rest of piece	Whole sample*
160	Treated	61.9	39.5	46.6	52.8	31.2	41.2
	Control	34.2	29.2	30.4	32.8	27.7	30.4

* Corrected for size of sample in the two portions. In pieces of different size a layer of equal thickness will represent a different proportion of the total weight. Thus in the large pieces above the first layer made up 28% of the total weight of the pieces, while in the smaller pieces a layer of approximately equal thickness represented 48% of the pieces.

DISTRIBUTION OF CHLORIDE AFTER DECOMPOSITION OF THE CHLORHYDRIN

In peeled tubers. Analyses of the portion of the tubers near the cut surface for total chloride after the decomposition of absorbed chlorhydrin has shown that a large part of the additional chloride due to the chlorhydrin is found in this region. Thus in a preliminary experiment in which juice was obtained from four portions of pieces which had been treated with ethylene chlorhydrin vapor, and which had been stored until no unaltered chlorhydrin was recovered upon distillation, the total chloride present expressed as cc. N/10 chloride per 25 cc. juice was as follows: outer layer, 15.8; second layer, 9.9; third layer, 10.4; inner portion, 10.2. The juice from the outer layer contained about 50 per cent more chloride than that from the remaining portions of the pieces in which the chloride distribution was uniform. The chloride distribution in treated tubers was

therefore further investigated together with that in control pieces which had been handled the same way as the treated except for the exposure to the chemical.

Data covering determinations made on some pieces which had been treated by the soak method are given in Table VIII. These tubers on analysis yielded the equivalent of 12.0 cc. of 0.1 molar chlorhydrin per

TABLE VIII

DISTRIBUTION OF CHLORIDES IN POTATO TUBERS AFTER THE DECOMPOSITION OF THE ABSORBED CHLORHYDRIN*

<table>
<tr><th rowspan="2">Chloride content, cc. 0.1 molar</th><th rowspan="2">Treated or control</th><th colspan="4">Portion of tubers</th><th rowspan="2">Whole sample**</th></tr>
<tr><th>Outer layer</th><th>Second layer</th><th>Third layer</th><th>Inner portion</th></tr>
<tr><td>Per 25 cc. juice, 9 days after treatment</td><td>Treated
Control</td><td>16.7
10.7</td><td>10.2
7.1</td><td>10.4
7.3</td><td>9.6
7.5</td><td>12.5
8.3</td></tr>
<tr><td>Per 100 g. fresh wt., 11 days after treatment</td><td>Treated
Control</td><td>57.6
28.4</td><td colspan="2">35.2
22.0</td><td>30.8
21.7</td><td>40.0
24.2</td></tr>
<tr><td>Per 100 g. dry wt., 11 days after treatment</td><td>Treated
Control</td><td>237
117</td><td colspan="2">143
89</td><td>135
95</td><td>169
101</td></tr>
</table>

* Cut pieces soaked for one hour in a solution containing 20 cc. of 40% chlorhydrin per l.; controls soaked in distilled water. Chlorhydrin content immediately after treatment was equivalent to 12.0 cc. 0.1 molar chlorhydrin solution; 7 days after treatment, 0.15 cc. 0.1 molar solution.

** Values corrected for differences in weight of the various layers.

100 grams of fresh tissue immediately after treatment and 7 days later only 0.15 cc. could be obtained from 100 grams of tissue. The chloride content based on juice obtained from four layers of both treated and control tubers 9 days after treatment and chloride analyses of the pieces divided into three layers based on both fresh and dry weight 11 days after treatment are given in the table. The data show that the additional chloride present in the treated tubers is not distributed evenly throughout but that the outer portion of the tubers contains about twice as much of the extra chloride as the rest of the tissue. The suberized layer which forms at the cut surface is very thin and the moisture determinations showed that the higher chloride content obtained in the expressed juice was not due to a lower moisture content in the portion peeled for the first layer.

The chloride distribution after the decomposition of the absorbed chlorhydrin was also determined in the experiment on the effect of cutting on the disappearance of chlorhydrin reported in Table VII. In these experiments the quantity of additional chloride in the outer layer was found to be from 2.7 to 4.8 times that present in the other portions of the treated pieces.

Although the chlorhydrin becomes equally distributed throughout the

tubers soon after treatment there is a period of a number of hours during which the outer layer contains considerably more chlorhydrin than the rest of the piece. Thus the high chloride concentration at the surface might be due to the decomposition taking place while this outer layer contains so much more chlorhydrin. It was therefore desirable to determine the chloride content sooner after the end of treatment and without waiting for the complete decomposition of the chlorhydrin. This was done in the experiment reported in Table IX. As a matter of fact the chlorhydrin decomposi-

TABLE IX

DECOMPOSITION OF ABSORBED CHLORHYDRIN AND DISTRIBUTION OF CHLORIDES IN TUBERS TREATED BY THE SOAK METHOD

Hours from end of treatment	Determination made	Treated or control	Chloride or chlorhydrin content, cc. 0.1 molar per 100 cc. juice				
			Outer layer	Second layer	Third layer	Inner portion	Whole sample*
0	Chlorhydrin	Treated	39.8	22.2	6.4	1.0	17.9
	Total chloride	Control	46.0	48.6	46.6	46.6	47.0
21	Chlorhydrin	Treated	18.3	18.0	17.4	14.6	16.7
	Total chloride	Treated	68.0	65.8	62.8	59.0	62.8
	Total chloride	Control	47.8	45.2	44.0	43.0	44.6
93	Chlorhydrin	Treated	5.6	6.9		7.4	6.6
	Total chloride	Treated	74.3	54.8		57.3	63.1
	Total chloride	Control	52.3	43.0	43.0	45.8	46.1
93	Chloride from decomposed chlorhydrin**	Treated	15.4	8.1		10.2	7.9

* Corrected for differences in weight of the layers taken for extraction of juice.

** These values were obtained by subtracting from the total chloride content the chloride due to unaltered chlorhydrin and the chloride content of the control at this sampling. Correction is made for estimated 85% recovery in determination of chlorhydrin.

tion is not very rapid immediately after treatment as is shown in Tables III and IV. In the experiment shown in Table IX only a small amount of chlorhydrin was decomposed during the first 21 hours. However, to further eliminate any advantage the first layer had had due to increased chlorhydrin content at the start, this layer was removed from all the tubers at the 21-hour sampling period and thus the layer which now became the first layer for the 93-hour sample had previously been the second layer. The results show that in spite of this precaution and after allowing for the somewhat higher chloride content at the surface of the control pieces the chloride due to the decomposition of the chlorhydrin is over 50 per cent higher at the cut surface than in the rest of the piece.

In whole tubers. Not as many experiments were carried out with whole tubers as with peeled tubers due to the more difficult sampling problem,

TABLE X

DECOMPOSITION OF ETHYLENE CHLORHYDRIN IN VAPOR-TREATED WHOLE TUBERS AND SUBSEQUENT CHLORIDE DISTRIBUTION WITHIN THE TUBERS

Hours from end of treatment	Chlorhydrin present, cc. 0.1 molar per 100 g.			Chlorhydrin lost as vapor, cc. 0.1 molar per 100 g.		
0	8.1			—		
74	4.1			0.33		
122	3.1			0.12		
190	0.3			0.08		
	Cc. 0.1 molar chloride per 100 g.					
	Treated or control	First layer	Second layer	Third layer	Inner portion	Whole sample*
238	Treated	42.2	31.4	31.6	30.9	35.1
	Control	27.2	23.1	26.1	26.5	25.6

* Values corrected for differences in weight of the various layers.

TABLE XI

DECOMPOSITION OF ABSORBED CHLORHYDRIN AND DISTRIBUTION OF CHLORIDE IN WHOLE TUBERS

Hours from end of treatment	Determination made	Treated or control	Chloride or chlorhydrin content, cc. 0.1 molar per 100 cc. juice				
			Outer layer	Second layer	Third layer	Inner portion	Whole sample*
0	Chlorhydrin	Treated	26.7	20.8	16.6	12.0	18.6
	Total chloride	Treated	81.4	64.8	56.3	50.7	62.4
	Total chloride	Control	46.4	38.4	34.0	34.0	38.1
72	Chlorhydrin	Treated	11.2	10.8	12.0	12.0	11.5
	Total chloride	Treated	72.8	60.4	54.4	52.8	60.5
	Total chloride	Control	49.0	39.0	35.2	38.0	40.5
120	Chlorhydrin	Treated	4.6	5.6	5.8	6.6	5.6
	Total chloride	Treated	66.0**	51.0**	45.6**	44.8**	52.2**
	Total chloride	Control	51.0	40.2	37.2	39.2	42.1
0	Chloride from decomposed chlorhydrin†	Treated	3.6	2.0	2.8	2.6	2.8
72			10.6	8.7	5.1	0.7	6.3
120			9.6	4.2	1.6	0	3.9

* Corrected for small differences in weight of the four layers taken for extraction of juice.

** Some injury was evident from the treatment at the second and third sampling periods and the samples taken consisted only of uninjured tubers. As a result the analyses show that the sample at the third sampling period had absorbed less chlorhydrin than the average of the whole treatment; the tubers which had taken up the largest amount of chlorhydrin had been eliminated because they showed injury. This sample is included however because it retains only a small amount of unaltered chlorhydrin and shows the unequal distribution of the chloride resulting from the chlorhydrin decomposed.

† These values were obtained by subtracting from the total chloride content the chloride due to unaltered chlorhydrin and the chloride content of the controls. Correction is made for estimated 85% recovery in chlorhydrin determinations.

but in so far as the experiments have gone the data show that in whole tubers also more of the chloride which results from the decomposition of the absorbed chlorhydrin is found in the outer layer. Very small whole tubers were used to obtain the data shown in Table X and thus a large number of tubers could be taken for each determination and individual variation largely eliminated. The extra chloride was found to be over twice as high in the outer layer as in the rest of the tuber. In another experiment (Table XI) the chlorhydrin and total chloride content of the expressed juice from four layers of whole tubers were followed at intervals after treatment. In this experiment also more of the chlorhydrin chloride was found in the outer layer.

DISCUSSION

The data in Tables VII, VIII, IX, and XI show that in the controls too somewhat larger amounts of chloride are present in the outer layer than in the rest of the tuber although this increased chloride is proportionately much less than with the treated samples. It is possible that this extra chloride at the surface of the control pieces may arise from the fact that the tubers lose some water during the experimental period and that in the movement of water from the interior to the surface some chlorides are carried along and of course are left behind when the water evaporates. That the higher chloride content found at the surface is not due to a lower moisture content in this layer is shown by the analyses reported in Table VIII. In any event, although the evidence presented in this paper indicates that in treated tubers there is probably more active decomposition of chlorhydrin at the cut surface and that this accounts for most of the extra chloride found in the first layer, the possibility of another explanation for the increased chloride content at the surface of treated tubers is not entirely excluded until the factors causing the increased chloride at the surface of control tubers are known and the extent to which these factors operate in the treated tissues have been determined. Since the decomposition of the absorbed chlorhydrin is apparently brought about by the metabolic activity of the tubers and since cutting increases the rate of decomposition, one would expect increased decomposition at the cut surface which is known to be the seat of intense metabolic activity (11). Similarly the outer regions of tubers with peel intact are probably also more active metabolically than the interior.

It will be noted that the amount of chlorhydrin taken up as estimated by the increase in the total chloride content is always somewhat higher than that given by distillation at the end of the treatment period (see Tables VII and VIII, for example). This would be expected since the distillation method is known to give only about 85 per cent recovery. Other factors which may cause the value for total chloride content to be some-

what higher are the decomposition of some of the chlorhydrin during the treatment period and the loss of some moisture in the interval from the end of the treatment to the time when the total chloride content is determined.

Planting tests have shown that when whole tubers are treated with ethylene chlorhydrin better results are obtained if the tubers are stored for a few days after the end of treatment than if they are planted at once (5). Experiments reported in the present paper show that if tubers are cut into pieces and planted the absorbed chlorhydrin decomposes more rapidly than in the whole tubers from the same treatment which are stored in air during the interval. The advantage of storing the treated tubers for a short period before planting may therefore be due to the fact that in such tubers a higher concentration of chlorhydrin is maintained for a longer time than when planted at once.

SUMMARY

Periodic analyses of potato tissue which has absorbed ethylene chlorhydrin show that the chlorhydrin is rapidly decomposed in the tissue although chlorhydrin is relatively very stable in expressed potato juice or in buffers at the pH of treated potatoes. About one-fifth or less of the absorbed chlorhydrin is given off unaltered in the vapor form and the rest is decomposed in the tissue at a rate varying from about 0.3 to 4.0 mg. per 100 g. of tissue per hour.

Increasing the amount of cut surface in potato tissue containing chlorhydrin increases the rate of decomposition and subsequent determination of the chloride content of the pieces shows that a large part of the chloride which arises from the chlorhydrin is found near the cut surface. These results indicate that the decomposition of chlorhydrin is more rapid at the cut surface. Analyses of whole tubers after the decomposition of the absorbed chlorhydrin show that in whole tuber treatments also a large proportion of the additional chloride is found in the outer region of the tubers.

LITERATURE CITED

1. BOZZA, GINO, e GIUSEPPINA GALLARATI. Ricèrche sulla clorindrina etilenica II. Giorn. Chim. Indus. ed Applic. **13**: 163–173. 1931.
2. BROOKS, BENJAMIN T. Ethylene and propylene chlorohydrins and glycols from oil gas. Chem. & Met. Eng. **22**: 629–633. 1920.
3. DENNY, F. E. Hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 118–125. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 59–66. 1926.)
4. ——— Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 386–396. 1926. (*Also in* Contrib. Boyce Thompson Inst. **1**: 169–180. 1926.)
5. DENNY, F. E., and LAWRENCE P. MILLER. Further experiments on shortening the rest period of potato tubers. Contrib. Boyce Thompson Inst. **7**: 157–181. 1935.

6. EVANS, W. P. Über die Abspaltungsgeschwindigkeiten von Chlorwasserstoff aus Chlorhydrinen und ihre Beziehung zur stereochemischen Konstitution. Zeitschr. Physik. Chem. **7**: 337–357. 1891.
7. GUTHRIE, JOHN D. Metabolism of citric, sulphuric, and nitric acid in the potato tuber. An explanation for the high pH of the juice of tubers treated with ethylene chlorhydrin. Contrib. Boyce Thompson Inst. **6**: 247–268. 1934.
8. MILLER, LAWRENCE P. The effect of treatments with ethylene chlorhydrin on the pH of the expressed juice of potato tubers. Contrib. Boyce Thompson Inst. **3**: 321–335. 1931.
9. MILLER, LAWRENCE P., and F. E. DENNY. Relation between quantity of ethylene chlorhydrin absorbed and growth response in treatments for shortening the rest period of potato tubers. Contrib. Boyce Thompson Inst. **8**: 121–136. 1936.
10. SMITH, LENNART. Der Reaktionsmechanismus bei alkalischer Chlorhydrinzersetzung. Zeitschr. Physik. Chem. Abt. A. **152**: 153–156. 1930.
11. STEWART, F. C., R. WRIGHT, and W. E. BERRY. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water, with observations upon surface: volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.
12. SUNDERMAN, F. WILLIAM, and PRISCILLA WILLIAMS. The analysis of chloride in tissues. Jour. Biol. Chem. **102**: 279–285. 1933.

ANATOMICAL STUDY OF ROOT PRODUCTION ON APPLICATION OF INDOLEBUTYRIC ACID TO CISSUS AERIAL ROOTS

Norma E. Pfeiffer

Among the plants already tested for gross reaction to synthetic growth substances are tropical species of *Cissus* which produce aerial roots ordinarily remaining unbranched until striking a substratum. Laibach (7) in 1933, working with *Cissus gongyloides* (Forsk.) Vahl, treated a strip of 1 to 1.5 cm. either at the tip or 2 cm. back, with his then-new growth substance paste and observed within five hours concave curvature on the side of application. Andreas (1) in 1937 found that the aerial roots in *Vitis gongyloides* (presumably the same form as Laibach used) had a growth zone of 30 to 50 cm. in length, that they grew 12 to 14 cm. in 24 hours, and that growth by day was distinctly greater than at night, the reverse of an earlier report on *Cissus* roots by Blaauw (2); Andreas concluded that temperature was the limiting factor. He found that various substances as orchid pollinia, rhizopin and hetero-auxin, retarded the growth of air roots and brought about positive curvature, with as much as 90° recorded for one concentration of indoleacetic acid in lanolin.

Earlier Zimmerman and Hitchcock (10) used various concentrations of six synthetic growth substances (either in water solution or in lanolin) on roots of *Cissus sicyoides* L. var. *Jacquini* Planchon. When intact roots were treated with a lanolin paste of the substance, either at the tip or along the region of elongation, new branch roots appeared through the epidermis in three to five days; the elongation of the main root was retarded and swelling occurred. Further growth of these new roots was inhibited by the resumption of growth by the main root, but elongation continued with re-treatment of the main tip. New lateral roots above the cut surface are also produced when roots are severed, as previously reported by Moore (8) and much earlier for another *Cissus* species by Blaauw (2).

In a very recent and excellent discussion of results obtained with growth substances to date, Jost (5) has summarized the work done on root development. He states that the development of the primordia of side roots is tied up with growth and division of cells but that not every cell increase is to be considered as leading to root development.

Since no account of the origin and development of lateral roots as a result of the use of synthetic growth substances has been noted in the literature, the roots of *Cissus sicyoides* L. var. *Jacquini* Planchon were selected as favorable material for an anatomical study of such development. The comparative effectiveness of the different compounds has already been considered by Zimmerman and Hitchcock (11), who report

α-naphthaleneacetic acid and its salts as more effective in initiating roots than the indole compounds. Alpha-naphthaleneacetic, indolebutyric, indoleacetic and indolepropionic acids were used in connection with this anatomical study. The description deals mainly with a more complete series of roots treated with indolebutyric acid, which is more effective than indolepropionic acid and is less apt to show toxic effects than the other two compounds mentioned.

METHODS

Actively growing whole roots were treated either at the tip or in zones definite distances back of the tip with lanolin containing the chemical substance. In a few cases, the epidermis was scraped for a short distance with a sharp razor blade, and the lanolin mixture applied at the wound, which was 1.5, 3, or 10 cm. back from the tip.

After definite intervals, the treated material was examined in the gross and collections made for preserving for permanent mounts. Various fixing agents were used, including Flemming's weaker, formalin-alcohol with and without acetic acid, and an aqueous picro-acetic acid solution. Material was imbedded in paraffin. Longitudinal and transverse sections were stained in crystal violet and safranin, in Haidenhain's haematoxylin, less frequently in Delafield's haematoxylin or safranin and light green. Free-hand sections were made at the time of preserving for study of the fresh tissues and for microchemical tests.

The deeply staining contents of the outermost layers, the number of raphides produced and the large amount of mucilage exuding from the crystal-bearing cells as well as from smaller mucilage canals near the xylem cells, present a problem in making clear neat preparations either in fresh or paraffin mounts. Washing in water and the addition of a drop of dilute hydrochloric acid helped to clear fresh sections. Dilute chloral hydrate solution decolorized the cells and was rather ineffective in clearing. Among the killing agents, Flemming's weaker was most satisfactory for nuclear division and cell contents in general. The usual proportions in formalin-alcohol often resulted in plasmolysis, as did the picric solution which, in addition, distended the crystal cells greatly at the same time that it cleared them of mucilage. Cellulose particles were readily distinguished in preparations of roots killed by means of each of the three reagents; formalin-alcohol was very favorable in this regard. No stain combination was found which was effective for other tissues when the outermost cells were satisfactorily light.

RESULTS

The aerial roots in this variety of *Cissus sicyoides* contain anthocyanin in the outermost layers in the younger regions except at the lighter somewhat blunt tip, to which a small brown dead remnant of the cap may

adhere. Many of the cells contain chloroplasts; beyond the anthocyanin-bearing portion, the root therefore appears distinctly green until the brown cork layers develop. Lacking root hairs, the root appears smooth superficially, and only with magnification do numerous small lenticel-like structures appear. Longitudinal sections show that the cap proper extends for some distance back over the growing tissues, and that it shows the apical differentiated region known as the columella. The roots resemble those of other tropical vines of the same family described by Goebel and Sandt (4).

The vascular system is usually tetrarch or pentarch, although there may be six, seven or eight xylem strands, with alternating phloem groups. The pericycle is several-layered where it lies over the xylem (Fig. 1 A) but consists of a single large-celled layer where adjacent to the phloem (Fig. 2). The endodermis is distinguished by its contents in the younger regions, and in the older portions by modified walls as well. The cortex consists of ten or more layers of cells in which there are usually two series of crystal cells which secrete much mucilage. The epidermis and one or more hypodermal layers contain anthocyanin and much deeply staining material. The differentiated tissues are apparently much like those briefly described for *Cissus velutinus* by Goebel and Sandt (4). In general, similar anatomy has been described by Turner (9) for the root of *Vitis rotundifolia* except that he designates the layers of cells lying immediately over the protoxylem as parenchyma and considers that there is only one pericyclic layer in this region.

It has been previously pointed out in *Cissus velutinus* and other forms (4) that the part in the light becomes green when a root enters the soil; decapitation has the same effect. The same result is seen here after treatment with synthetic growth substances. Furthermore elongation is retarded by any one of these methods, and the root increases in diameter. A cumulative effect may be obtained in the degree of swelling by application of growth substance and subsequent covering of the root with peat.

The concentrations of indolebutyric acid used on intact roots ranged from 10 mg. of the chemical to 1 g. of lanolin as the strongest to 1 mg. per g. lanolin as the most dilute. Other roots lightly scraped to remove the outermost layers for a short distance, and treated with lower concentrations (0.5 and 0.1 mg.) are reported here. An endeavor was made to apply small amounts of lanolin paste.

The first noticeable effects, aside from the feel of the treated root, which gives the effect of a state of rigor rather than ordinary turgor, are seen in the protoplasm of the living cells of the phloem and in the pericycle cells adjacent to the xylem. These cells enlarge and there is an increase in the amount of staining material which is noticeable even within three hours. Later microchemical tests show that there is an increase in sugars.

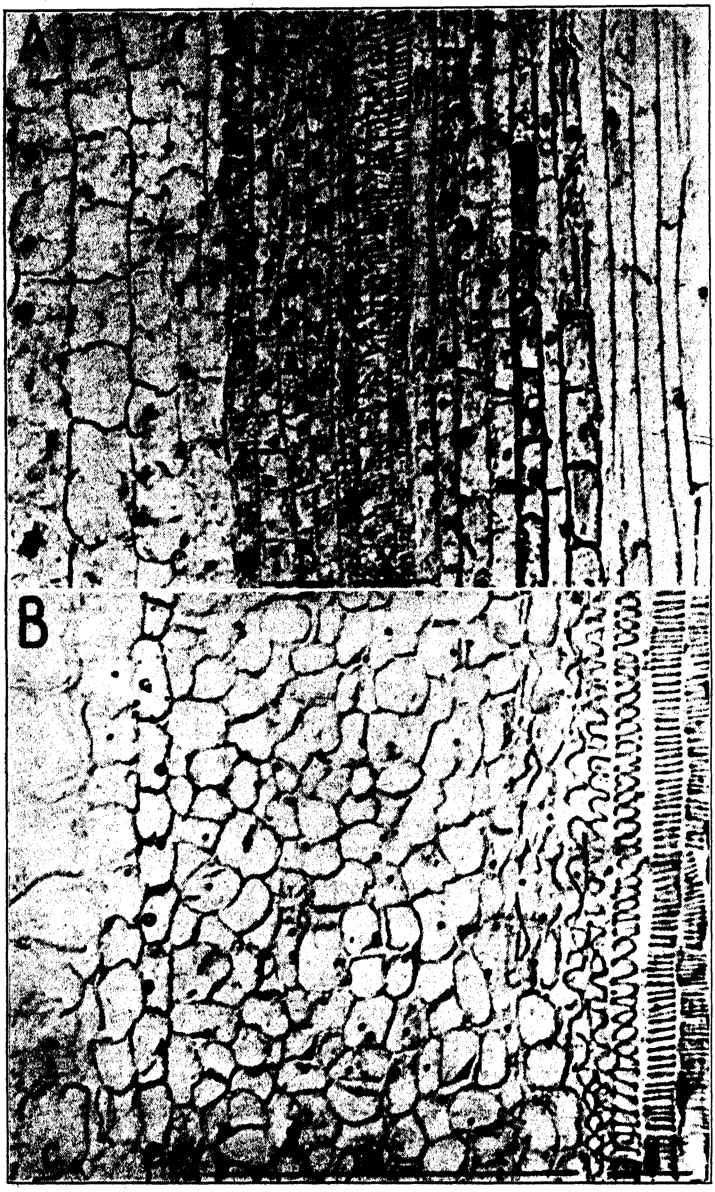


FIGURE 1. Longitudinal sections of roots showing portion of xylem, pericycle, endodermis and cortex. A. Control with few pericycle layers. Magnification 305. B. Four days after treatment of a zone with indolebutyric acid, with many pericycle layers; *c*, cortex; *e*, endodermis; *p*, pericycle; *x*, xylem. Magnification 300.

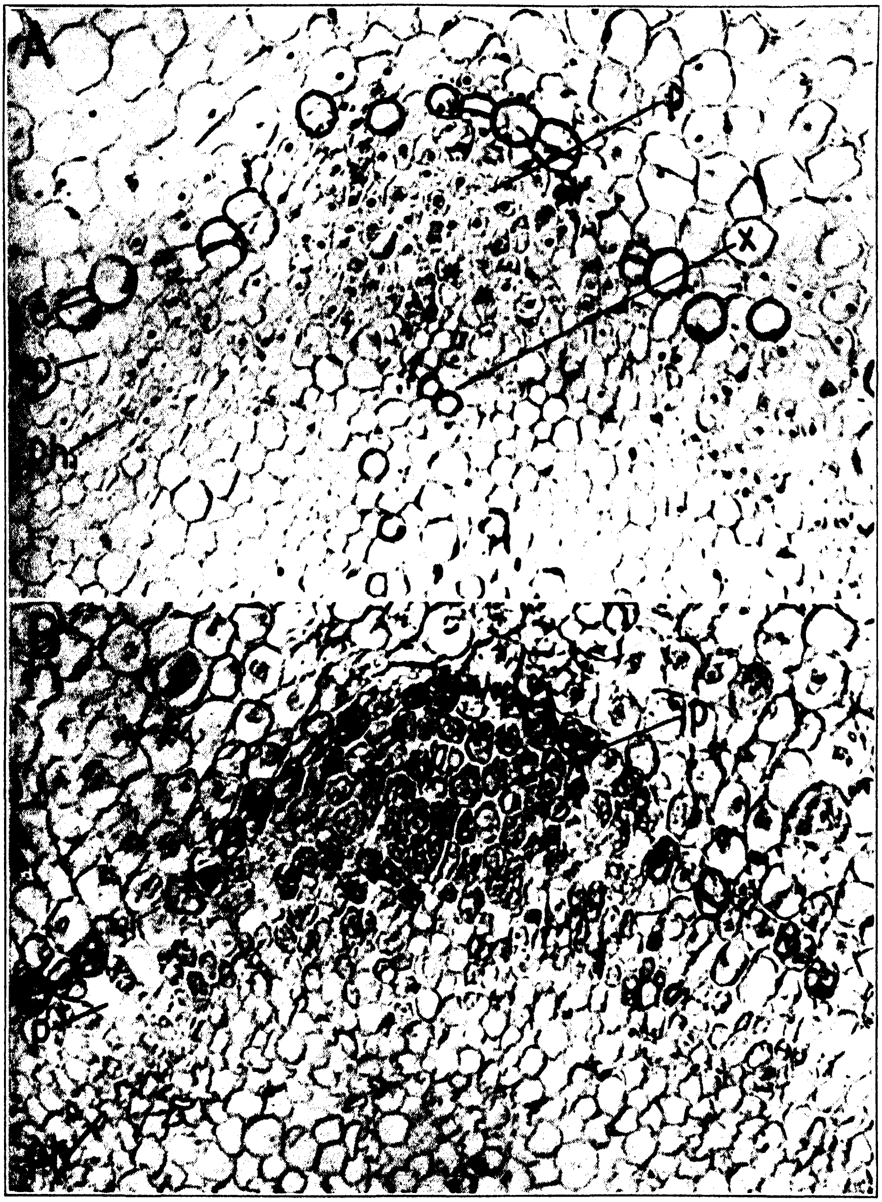


FIGURE 2. Roots treated at tip with indolebutyric acid, 10 mg. per g. lanolin. A. Twenty-four hours after application; plane of young walls as well as spindles indicate periclinal divisions in pericycle. B. Twenty-seven hours after application; pericycle cells have undergone periclinal and anticlinal divisions; *ph*, phloem. Magnification 300.

Periclinal divisions of cells in the pericycle adjacent to the xylem soon occur (Fig. 2) and there is cell division in the inner phloem cells and especially at the lateral ends of the group abutting on the parenchyma cells which separate the phloem from the xylem strand (Fig. 3 *ph*). In the pericycle, the first divisions occur without reference to any particular layer. Cellulose particles are prominent in these active cells, both in living material and in stained preparations. Nuclear division is accompanied by formation of cell walls on normal spindles. These membranes are thin and at least at first are readily distinguished from the walls of the original cells, as may be seen in the pericycle in Figure 2 B showing the condition 27 hours after treatment with 1 per cent indolebutyric acid paste. By this time, an occasional endodermal cell may have divided. Many of the cells in this layer now show thicker walls. All tissues have a higher water content, which results in a distended effect of the cells when seen in cross section. This is particularly evident in the cortex and crystal cells. Thin-walled cells especially are easily plasmolyzed by a killing and fixing agent which usually gives good results.

As time goes on, the parenchyma cells about the xylem, the innermost cortical cells and the large central pith cells frequently show enlarged nuclei which may divide when the higher concentrations (1 or 0.5 per cent) of indolebutyric acid are used. Because the cells do not elongate vertically as in untreated roots and because the new walls are thin, it frequently appears in cross section as if some of the parenchymatous cells were binucleate. In comparing longitudinal sections, there is no evidence for such a condition. Cells in all stages of division of the nucleus and subsequent development show a normal course of spindle and wall formation, with no deviation in chromosome behavior. Even with such marked development of pericycle tissue and of radially elongated cortical cells as is brought about with the use of an indoleacetic acid mixture, there was no indication of failure to develop walls. It must be emphasized that the concentrations used are decidedly lower than those applied in recent investigations on decapitated bean stems, where Kraus, Brown and Hamner (6) found a multinucleate condition in many cells near the point of application of the mixture. In the roots, the effort was made to avoid injury rather than to provide the maximum of stimulation to the tissues. There were frequently nuclei with two or three nucleoli, but this occurs in this form also in untreated roots.

The pericycle cells over the phloem become elongated in a radial direction, but less frequently divide. Those adjacent to the xylem strands keep the lead in division and produce rounded masses of tissue as shown in Figure 4 A and B, where the root primordia resulting from application of α-naphthaleneacetic and indoleacetic acids are similar to those appearing after treatment with indolebutyric acid. The activity is not uniform at all

the points, and in any single cross section one pericycle mass may show relatively few tangential divisions while another may have already produced a fair sized root primordium. In tracing through a series of cross sections, some of the pericyclic points may be consistently more active than others, giving rise to a vertical series of root primordia. Sometimes a band of pericycle cells develops with no primordia evident (Fig. 1 B). This lack of uniform development is also seen in longitudinal sections as well as later when the roots emerge. It is true that some treatments, as a higher concentration, or a second application, or a combination of treatment with a chemical substance and imbedding in peat, give rise to more primordia, which are necessarily closer to each other (Fig. 5 A) than the fewer roots resulting from single applications of lower concentrations. There is apparently an adequate food supply in the plant for a very large number of roots, as shows also when the root strikes a substratum (Fig. 5 B).

Division occurs earlier in the region nearest the point of application, but progresses gradually from that point. Figure 3 A shows a section in the apical centimeter of a root treated 31 hours earlier; a number of periclinal divisions followed by anticlinal divisions, have resulted in a fairly conspicuous mass of pericyclic tissue. Figure 3 B shows the situation about 2 to 2.5 cm. back, where the cells have increased in size, but few divisions have occurred. Since there is much variation between individual main roots, no attempt was made to determine accurately how far the influence of the applied growth substance can be traced microscopically.

Later stages in the development of lateral roots are shown in Figures 4 C, 5, and 6. It is clear in following the endodermis, which now contains deeply staining material, that the tissue giving rise to the root lies entirely within it. The roots arising as a result of the application of synthetic growth substances come from division of the pericycle layers lying over the protoxylem points in the same fashion as the laterals arising from the striking of the main root into a substratum (Fig. 5 B). The length of time required for development is dependent in large part on the concentration used and on the specific chemical as well as on the individual root.

With a second application of the growth substances, the pericyclic tissue may be so stimulated as to give a practically continuous band of meristematic cells. Here so many primordia arise that they are not separate except at the tips, but emerge as a sort of flattened flange of tissue, as previously reported (11, p. 340). This, however, shows a number of vascular cylinders, sometimes not wholly distinct, but indicating that the tissue of a number of roots is connected. This is considered a definite abnormality as compared with the primordia chiefly discussed and is doubtless related to the larger amount of growth substance applied.

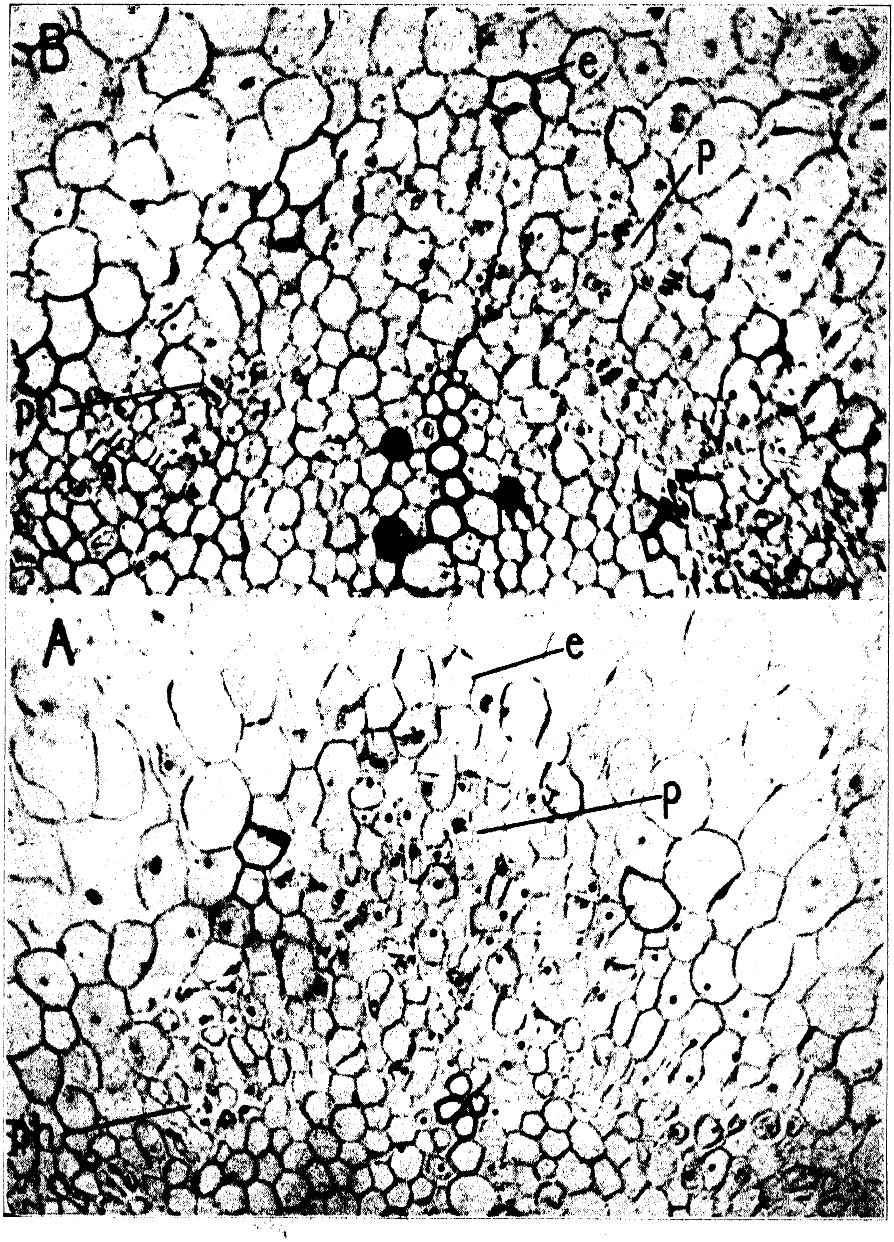


FIGURE 3. Root treated like that in Figure 2, 31 hours after application. A. Cross section within few mm. of tip. B. Similar section in zone about 2 to 2.5 cm. back of tip. Magnification 300.

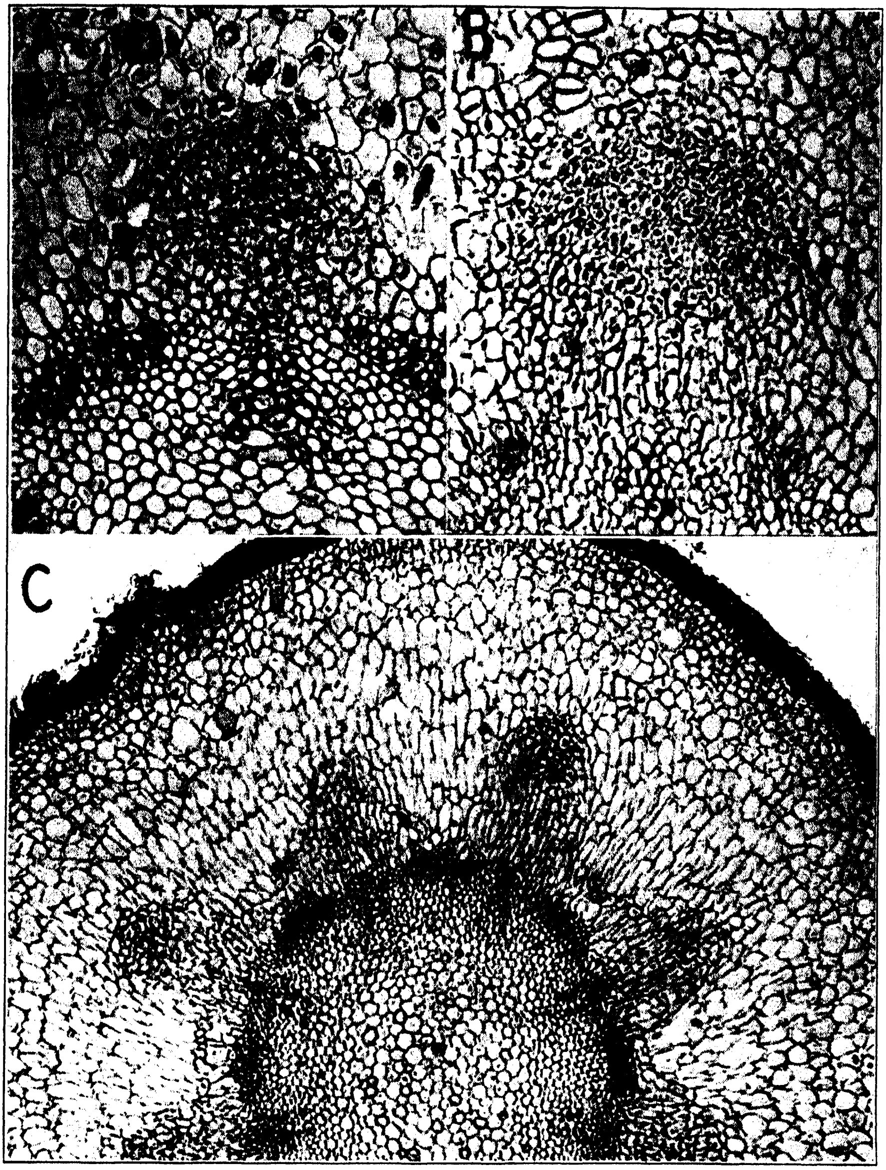


FIGURE 4. Intermediate stages in development of root primordia resulting from application of synthetic growth substances. A. Indoleacetic acid, 5 mg. per g. lanolin, 2 days. B. α-naphthaleneacetic acid, 0.5 mg. per g. lanolin, 4 days. Magnification ca. 135. C. Indoleacetic acid, 2.5 mg. per g. lanolin, time not recorded. Magnification 60.

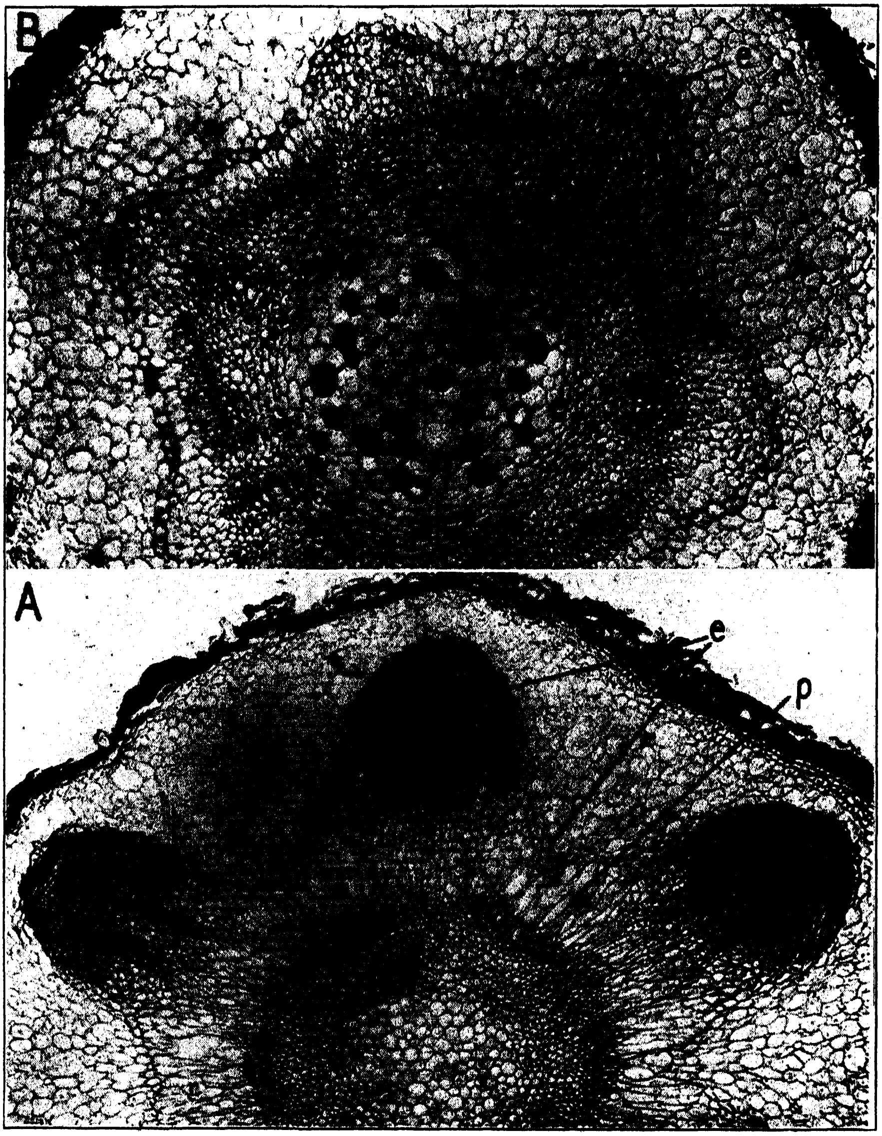


FIGURE 5. A. Cross section of root two days in peat subsequent to four days elapsed after application of indolebutyric acid (10 mg. per g. lanolin) to tip. Magnification 60. B. Root in peat two days. Magnification 100.

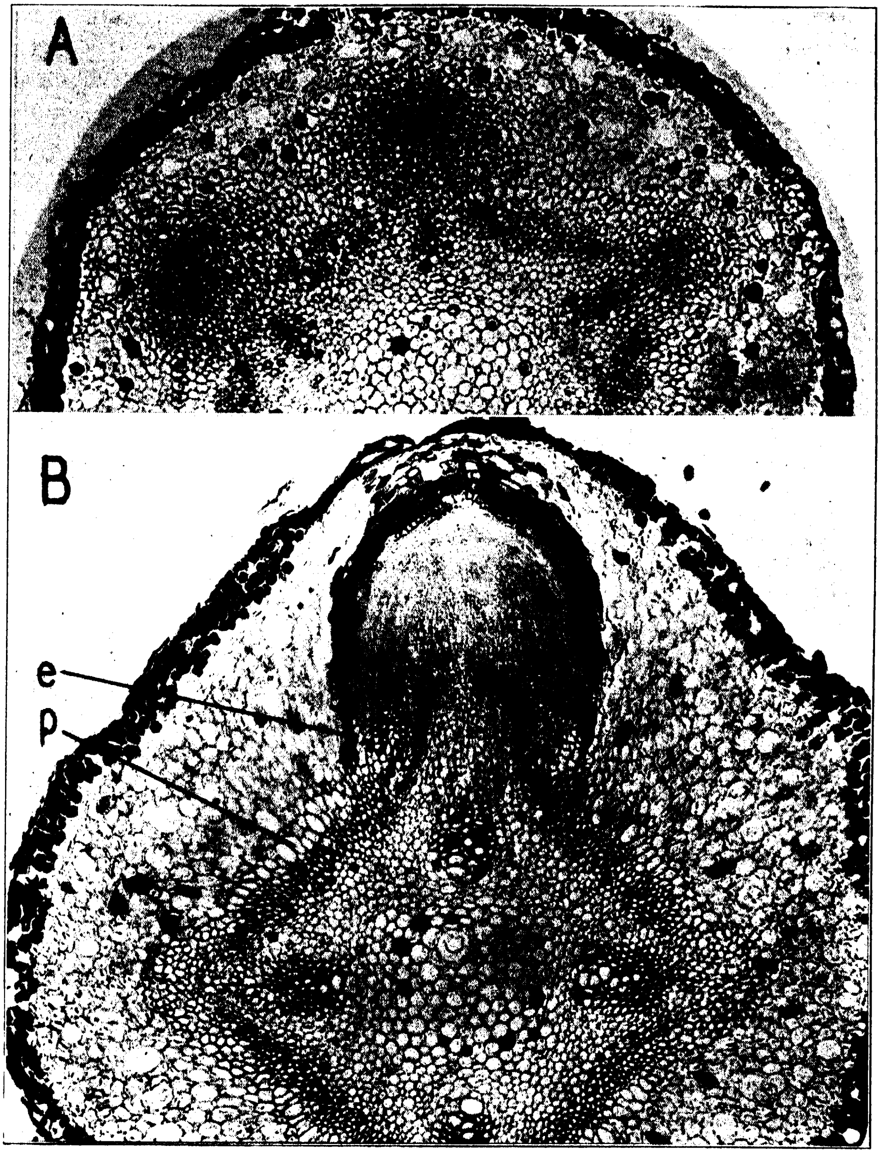


FIGURE 6. Cross section of roots four days after application of indolebutyric acid (10 mg. per g. lanolin). A. Less well developed primordia. B. Completely differentiated lateral root. Magnification 60.

DISCUSSION

Synthetic growth substances stimulate the development of lateral roots, which parallel that resulting from the striking of the root into a substratum. It is found that the selection of the specific growth substance and especially the use of low concentrations are of importance in producing these roots. The first results are increase in size and in amount of cytoplasm, as in any meristematic cells, although more vacuoles may appear here with the greater increase in water content of the treated tissues. With a supply of the synthetic substance intermediate between that which stimulates but few cell divisions and that which results in great proliferation of tissue, the course of development of the lateral roots from pericycle tissue proceeds with no indication of abnormalities in nuclear or cell division. With little growth substance, as is presumably the case farther away from the point of application, the situation may be that suggested by Jost (5) where increase in number of cells is brought about without subsequent development of a root primordium.

With a greater supply than is necessary for normal development of cells and tissues, the responses described by Kraus and his coworkers (6) may be found. The concentration used by them was three times as great as the most concentrated mixture in the present tests. Furthermore, with solutions of the highest concentration here used, the roots were usually intact, thus making a distinct barrier to easy direct diffusion of the substances, as compared with the cut surfaces of the bean stems. When the roots were scraped to break the epidermis, only small areas were exposed, and usually lower concentrations were used. It is therefore probable that less of the mixture as well as far lower concentrations were applied to those surfaces which are most nearly comparable to the cut stem surfaces. Certain it is that no such production of multinucleate cells or great proliferation of tissues occurred in *Cissus* roots.

The same comparisons may be made with the results of Borthwick, Hamner and Parker (3) who decapitated tomato stems and used 20 mg. of indoleacetic acid per g. of lanolin. This is still a relatively strong concentration for this reagent, although lower than that used by Kraus and his coworkers.

The present results, however, do agree with those found in bean stems in showing that the phloem tissue is definitely responsive to the growth substance. Changes are seen in this tissue as early as in the pericycle and there is some basis for believing that the path of transfer is through the phloem.

From the earlier account of Zimmerman and Hitchcock (10) in which roots were seen to emerge from the epidermis in three to five days, it appears that transfer is fully as rapid here as in the stem described by Kraus, Brown and Hamner (6). This is substantiated by the microscopic

study of the various tissues affected by the presence of the indole compounds used.

SUMMARY

1. Aerial simple roots of *Cissus sicyoides* L. var. *Jacquini* Planchon were treated with synthetic growth substances in lanolin in concentrations from 0.1 mg. to 10 mg. per gram of lanolin. The results of the application of these substances (α-naphthaleneacetic, indolebutyric, indoleacetic, and indolepropionic acids) were similar. The effects of the application of indolebutyric acid are described.

2. The lanolin mixture was applied either to the tips of intact roots, to definite zones back of the tip or to small lightly scraped areas in the growing region 1.5 or 3 cm. back from the tip.

3. The first result observed is an increase in the amount of cytoplasm and in the size of the living cells in the phloem and in the pericycle adjacent to the xylem. Simultaneous increase in water content in these tissues is great, often leading to conspicuous vacuoles.

4. Periclinal divisions in the pericycle increase the number of layers of cells lying over the xylem. Subsequent divisions, both periclinal and anticlinal, produce small masses of tissue over the protoxylem points. These constitute the lateral root primordia.

5. Increase in size of nuclei and cells, and their division occur also in other tissues, especially the inner cortex, the pith and the parenchyma lying radially between the phloem and xylem strands. Nuclear and cell division are normal with the low concentrations used.

6. Along with these changes, the diameter of the main root becomes greater, while elongation is retarded.

7. The main root upon striking a substratum produces lateral branches. In main roots treated with synthetic growth substances, primordia are initiated and differentiated in the same manner.

8. Abnormalities, such as the production of vertical extensions of tissue with several root vascular cylinders, sometimes result from a second application of growth substance.

LITERATURE CITED

1. ANDREAS, CH. HANS. Wuchsstoff und Wachstum der Luftwurzeln der *Vitis gongyloides*. Proc. Akad. Wetensch. Amsterdam Ser. Sci. **40**: 174–180. 1937.
2. BLAAUW, A. H. Das Wachstum der Luftwurzeln einer Cissus-Art. Ann. Jard. Bot. Buitenzorg **26**: 266–293. 1912.
3. BORTHWICK, H. A., K. C. HAMNER, and M. W. PARKER. Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. Bot. Gaz. **98**: 491–519. 1937.
4. GOEBEL, K. F., und W. SANDT. Untersuchungen an Luftwurzeln. (Botanische Abhandlungen. hrsg. von K. Goebel. Hft. 17.) 124 pp. Gustav Fischer, Jena. 1930.
5. JOST, L. Über Wuchsstoffe. Zeitschr. Bot. **31**: 95–121. 1937.

6. KRAUS, E. J., NELLIE A. BROWN, and K. C. HAMNER. Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. **98**: 370–420. 1936.
7. LAIBACH, F. Versuche mit Wuchsstoffpaste. Ber. Deutsch. Bot. Ges. **51**: 386–392. 1933.
8. MOORE, JOHN ADAM. Remarkable aerial roots of a tropical grape vine. Missouri Bot. Gard. Bull. **21**: 139–140. 1933.
9. TURNER, LEWIS M. Anatomy of aerial roots of *Vitis rotundifolia*. Bot. Gaz. **96**: 367–371. 1934.
10. ZIMMERMAN, P. W., and A. E. HITCHCOCK. The response of roots to "root-forming" substances. Contrib. Boyce Thompson Inst. **7**: 439–445. 1935.
11. ——— Comparative effectiveness of acids, esters, and salts as growth substances and methods of evaluating them. Contrib. Boyce Thompson Inst. **8**: 337–350. 1937.

INDEX

Date of issue.	Date of issue.	Date of issue.
18.5.40.		
18.7.40		
3.8.40		
27.8.40		
26.11.46		
17.8.47		
2.5.5		
29.1.57		
30.7.64		
14.1.65		
11.8.65		
19 SEP 1970		
1 SEP 1972		